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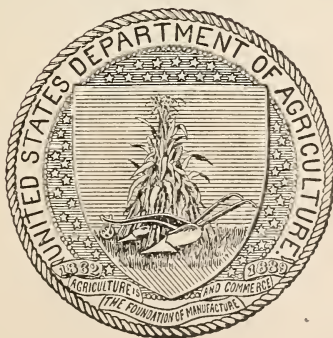
MILTON WHITNEY, Chief.

CERTAIN ORGANIC CONSTITUENTS OF SOILS IN RELATION TO SOIL FERTILITY.

BY

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ASSISTED BY J. J. SKINNER.



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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF SOILS,
Washington, D. C., June 10, 1907.

SIR: I have the honor to transmit herewith the manuscript of a technical paper entitled Certain Organic Constituents of Soils in Relation to Soil Fertility, by Oswald Schreiner, Howard S. Reed, and J. J. Skinner, of this Bureau. This article embodies the results of further experiments upon the effect of toxic organic substances upon soil conditions as indicated by the growth of plants in various cultures.

The material throws considerable additional light upon the question of soil fertility. In accordance with your suggestion it has been gone over carefully with Assistant Secretary Hays, who authorizes me to state that he concurs in my recommendation for its publication. This will form No. 47 in the series of bulletins of the Bureau of Soils.

Respectfully,

MILTON WHITNEY,
Chief of Bureau.

HON. JAMES WILSON,
Secretary of Agriculture.

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CERTAIN ORGANIC CONSTITUENTS OF SOILS IN RELATION TO SOIL FERTILITY.

INTRODUCTION.

The poor qualities of unproductive soils are not always due to deficiency in nutrient mineral substances, but are often due to the presence of substances actually deleterious to plant growth. The presence of such deleterious substances may be demonstrated by the properties which the soil and its aqueous extract possess. When an unproductive soil is extracted with water, the aqueous solution is found to possess the properties of the soil itself, so far as plant growth is concerned; when a good soil is extracted, a good soil extract is obtained, and a poor soil yields a poor soil extract. The plant-producing power of soil extracts follows that of the soils from which they were made, almost regardless of soil texture or geological origin; where this is not the case the unproductivity is probably due to a poor physical condition, hardpan or poor cultural methods. In many instances poor soil extracts produce plants which are far less developed than similar plants grown in pure distilled water under identical conditions. This toxicity of the soil extracts has been investigated quite thoroughly and numerous experiments reported in former bulletins of the Bureau of Soils, using the plant as an indicator.

The toxic properties can be removed from a soil extract by a number of quite simple operations, such as treatment with carbon black, ferric hydrate, and other absorbent agents, whereby the toxic material is apparently removed from the solution. This action seems to be quite general for all poor soil extracts, although in other respects the properties of the soil extracts differ appreciably. The toxicity of certain soil extracts can be destroyed by merely boiling them. This may be due in some cases to a destruction and in others to a volatilization of the toxic agents, in which latter case the toxic properties can be found in the distillate. The toxicity can also be much weakened by dilution with pure distilled water, so that in the weaker solution there may even result a stimulation in growth such as that commonly observed with weak solutions of poisons. These

toxic properties are also overcome in many instances by the addition of comparatively minute amounts of pyrogallol, naphthylamine, and other nonnutrient substances, a phenomenon which may be explained by assuming that these bodies have acted upon the toxic substance or have so altered the plant by stimulation or otherwise as to overcome them.

Another way of rendering the toxic properties innocuous is by the addition of certain of the fertilizer salts, as in practical agriculture in the field, or by the addition of calcium carbonate, which in the soil extract and in the soil itself is known to have very strong remedial properties where bad soil conditions exist. The addition of stable manure and green manure to the soil is also of the greatest benefit, as is well known in agricultural practice. Their beneficial action can not be entirely ascribed to the plant food or plant nutrients which these substances contain, but part of their action is due to the organic compounds which they contain and the changes which they set up in the soil.

It is, of course, well known that toxic soil conditions can be brought about by excess of salts or the presence of unfavorable inorganic compounds, such as ferrous iron, etc., but the properties of the toxic bodies causing unproductivity in ordinary agricultural soils under what might be termed normal conditions of continual crop culture, point strongly to the fact that the toxic bodies are organic and that the productiveness of a soil depends largely upon the condition of the organic matter in the soil and the processes which are at work in destroying the plant remains.

These toxic bodies, as shown in a former bulletin, may arise as the direct result of plant growth. When one crop succeeds another the soil is frequently rendered unfavorable for the support of a second crop of the same kind, even when the plants are only allowed to grow two or three weeks from the seed and can hardly have taken up enough plant food to have exhausted the supply naturally contained in the soil. Furthermore, the addition of plant nutrients to such a soil is not able entirely to overcome these toxic conditions for another crop of the same kind if immediately planted. They may, however, be overcome and the growth actually increased by the application of lime and green manure, either singly or in combination. The unfavorable conditions brought about by the excretion of material from the roots of the plant may affect the succeeding crop if immediately planted. When these plants are of a like species, such as wheat succeeding wheat, the effect is very marked. However, when cowpeas or corn is followed by wheat there is almost no detrimental effect, but when a closely related species like oats is used as the preceding crop the effect is again marked, although the retardation is not so great as when wheat follows wheat.

This same bad effect of root excretions is also shown by the effect of trees on wheat growing in conjunction with them, and is further illustrated by the poisonous effect which grass roots sometimes have on apple trees when sod is allowed to form in orchards.

Seeds will also throw off toxic substances during the process of germination which are detrimental to the growth of another crop of the same kind of seedlings, if planted before the unfavorable conditions have been ameliorated by treatment or by natural processes, such as oxidation or decay.

The beneficial effects of mineral plant nutrients and of organic manures are too well known to require more than brief mention in this place. When soil conditions are favorable for the decomposition and humification of organic material a marked increase in plant growth results. Numerous experiments showing this beneficial action have been described in Bulletins 28, 36, and 40 of this Bureau. A single experiment may be described in this place to illustrate the principle of this action.

Green-cowpea vines were added to a poor soil at the rate of 5,000 parts per million of soil. The soil was kept at optimum water content and frequently stirred during the next twenty-six days. As decomposition progressed the soil developed an odor like that of decomposed stable manure. At the end of twenty-six days green cowpea vines were added to another sample of the same soil in the same proportion as originally applied. Both the samples of soil were then immediately extracted with distilled water, and the extracts used as a medium for growing wheat plants in comparison with the extract of a sample of soil to which no cowpea vines had been added. At the end of eleven days the growth produced in each set of solutions was measured by taking the green weight of the plants. Representing the growth of the plants in the extract of untreated soil as 100, the growth in the extract made immediately after adding cowpea vines was 95, and that in the extract of soil in which cowpea vines had stood twenty-six days was 130. This shows that the application of green manure was able to produce an increased growth, amounting to 30 per cent where the plants grew so short a time as eleven days, provided that the green manure had had sufficient time to decompose.

The toxic properties of soils have been demonstrated and the existence of toxic bodies is a reality with which it is necessary to deal in future soil studies on the fertility and infertility of our agricultural lands. A very important phase of work in these soil studies yet remains to be done before a thorough knowledge of these bodies can be obtained and the best means of treatment to overcome them established. Many of their properties have already been determined, so far as they relate to plant growth. Not only have their

physical properties been studied, but the physiological properties, their effect on the growth and nutrition of plants or other physiological functions, have also been ascertained for many soils. It remains to make a closer study of the chemical properties of these toxic bodies and to isolate them from the soil.

PROPERTIES OF THE ORGANIC CONSTITUENTS OF SOILS.

The problem of isolating organic bodies from soils has been attempted from time to time in the history of agricultural science. Braconnot, Walser, Gyde, and others even attempted to show by chemical means the presence of the toxic excretions of plants, and it is largely due to their failure to obtain positive reactions that the matter was dropped for many years. Investigations have been reported from time to time on the isolation of various humic acids, crenic, apocrenic, ulmic, etc., but the composition of the acids isolated has been a most variable one, differing with each investigator and with each method employed. It would not be far from the truth to say that none of these acids have been shown to exist and that we know almost nothing about their chemical composition or constitution.

The work of isolating and studying the nature of such bodies is now in progress, but it is necessarily slow and difficult to accomplish. In the first place, the amount may be very small, and, secondly, the composition of the organic matter of the soil is undoubtedly very complex, being made up of many individual organic compounds. The absorptive power of the soil for organic bodies is so very great that the ordinary solvents, such as water, alcohol, chloroform, etc., take out but very minute amounts of organic material, although this material may be quite soluble when not associated with the soil. The amounts of organic matter in ordinary soils is really appreciably large, the average content being as high as 2.06 per cent for the soil and 0.83 per cent for the subsoil, as found in thousands of samples from all parts of the United States, covering in all 237 types of soil. It is obvious that this organic matter is of very complex composition, as its properties are quite different in soils from different localities. The amount of nitrogen also is considerable, as much as 0.1 to 1.0 per cent, and when we consider that only a small amount of this nitrogen is in the form of ammonium compounds or nitrate and that the larger amount is in organic form it is obvious that the problem is a very complex one, but one of great agricultural importance.

In view of the difficulties which are encountered, and must be encountered for some time, in the isolation of these bodies by purely analytical methods, it was thought that the synthetic way of adding

bodies of known composition, known properties, and known derivation from plants to culture media, in order to study their properties and the effect which they might have upon plant growth, would answer the question whether such bodies are toxic to plants much sooner than could possibly be done by first isolating them from the soil and then trying their properties. Proceeding in this synthetic manner, it is possible to reach a broader survey of the effect of organic plant remains and get a clearer conception of the manner in which these function in the soil.

Plant remains contain such organic compounds in their tissues as we have studied, hence it is not improbable that these exist in the soil as such, or as decomposition products, or as compounds formed from them by the action of aerobic and anaerobic micro-organisms. Prominent among these organic compounds would be the carbohydrates, proteids, and lecithins which exist in the living cells of every plant. During the decay of these complex organic bodies a large group of what are known as primary degradation products are formed and from these, secondary decomposition products may result.

Many of the normal decomposition products of proteids, such as leucine, asparagine, etc., are not toxic, but appear to serve as direct nutrients for the plants, but others, like tyrosine and products resulting from it, are inhibitive to plant growth. When proteids decompose under unfavorable soil conditions they may give rise to substances more toxic than the original; for example, phenols, cresols, and aromatic acids, which may also result from tyrosine. Choline, a decomposition product of lecithin, is moderately toxic to plant growth, but stimulates in low concentrations. Neurine results from the action of bacteria on choline and the widely distributed lecithin, and is moreover quite inhibitive to plant growth.

The cumarin mentioned below as occurring in certain plants and quite inhibitive to plant growth may also be formed from carbohydrates by the action of certain mold fungi. One of the fungi which occurs in certain soils has the ability to produce quinone from proteids. Small amounts of this quinone are harmful to plants and may injure growth unless it is converted into some harmless substance.

A fungus which causes decay of wood has the ability to form vanillin. This substance, which is toxic to plants, is commonly regarded as of rare occurrence, but is found in a number of common plants, and its derivatives may form a part of the organic constituents of the soil.

The presence of pyridine and related bodies in soils is rendered not only possible but extremely probable by recent soil investigations. Hence a study of their properties is of value in relation to soil conditions, and in general it may be said that pyridine and its compounds are distinctly toxic to plant growth.

Again, such substances as the terpenes are of interest in this connection because of their probable presence in soils. In general, it may be said that terpene compounds are inhibitive to plant growth. One of the terpene derivatives, borneol, which is quite toxic to plant growth, occurs in pine needles and in the common golden-rod. The terpene compounds are widely distributed and are to be regarded as plant excretions. Their introduction into the soil is almost inevitable through the decay of plants, many of which are agriculturally classed as weeds and known to contain terpene bodies. Since the terpene bodies, as is well known, are antiseptics, they would not themselves be materially altered by micro-organisms of the soil, although many of them are subject to oxidation. They would, therefore, be likely to persist in the soil in one form or another for some time. The presence of terpene derivatives in the soil is therefore more than probable and where they are persistent they may affect the growth of plants on that soil.

It has been demonstrated by the studies described in this bulletin that substances commonly used as fertilizers in agricultural practice have in addition to their function as plant nutrients a well-defined power to overcome and actually destroy toxic bodies. Substances like nitrate of soda and lime, acting in cooperation with the activities of the plant roots, are able to destroy or render harmless various organic substances which previously had a toxic effect on the plants. This fact is believed to be of distinct value in the study of soil problems, because it is known that many cases of infertility are simply due to the presence in the soil of compounds having a toxic action upon plants. When such soils are extracted with water the solution when used as a medium for plant growth displays many of the same characteristics as are displayed by solutions of the organic compounds described in this bulletin. Such toxic soil extracts may be greatly improved by the addition of fertilizer substances, but up to the present the method by which amelioration was brought about had not been demonstrated. It had been distinctly shown, however, that amelioration was not due to the addition of plant nutrients, since equal, if not greater, improvement resulted from treatments which added nothing in the nature of plant nutrients. The present investigations become of value, therefore, in showing that fertilizer substances have a power to act destructively upon deleterious organic compounds, especially when associated with the activities of growing plants.

Tyrosine, one of the degradation products of the proteids, has been found unfavorable to plant growth, a fact which is believed to shed light upon the first effects of green manures. When tyrosine is darkened by oxidation, it is changed in a manner analogous to the changes which occur during the decomposition of green manure. The dark-

colored solution that results from the oxidation of tyrosine is highly beneficial to plant growth and acts like an extract of well-rotted stable manure. Processes like this may occur during the decomposition and humification of organic matter in the soil.

The beneficial effects of processes which involve oxidation are well illustrated by the effects of neurine, choline, and betaine. Neurine, which is very toxic to wheat plants, is not a highly oxidized compound. Choline is more highly oxidized than neurine and is less toxic to plants. Betaine, which is still more highly oxidized than choline, is not at all toxic to plants in concentrations of 1,000 parts per million or less.

It is therefore readily understood how processes of oxidation may improve soil conditions when we know that toxic bodies like choline and tyrosine are converted into nontoxic bodies by oxidation. When the soil possesses the proper amount of water and a proper physical condition, it is possible for changes to occur in the organic constituents, which changes, commonly known as "humification," result in beneficial products. When the soil is saturated with water or is so packed that a sufficient supply of air is not afforded, then the organic matter may be differently decomposed, giving organic compounds in a low stage of oxidation which are unfavorable to plant growth, and bring about toxic conditions which temporarily restrict the productivity of the soil. When certain plants are used as green manures it has been found unwise to plant a given crop immediately, because their effect upon plant growth is not beneficial. After sufficient time has elapsed for decomposition to go on a beneficial effect is produced by such green manures. The immediate harmful effect is presumably due to the presence of bodies like tyrosine, cumarin, and their derivatives. Cumarin, which also has properties toxic to plants, is the odorous principle of many sweet-scented grasses and clovers. Processes of oxidation, which are known to occur in all fertile soils, convert such vegetable substances into beneficial humus like bodies.

THE EFFECT OF VARIOUS ORGANIC SUBSTANCES OF VEGETABLE ORIGIN.

Continued study of the phenomena exhibited by poor soils has shown that the deleterious properties are often due to the presence of toxic organic compounds. Accordingly, it seemed profitable to investigate the properties of a number of organic substances of vegetable origin with relation to the growth of higher plants. Many of the substances tried have been studied by others as sources of nutrition for bacteria and fungi, but the effect upon the higher plants has not been so extensively studied.

METHODS.

The experimentation consisted in studying the effect of a number of organic compounds upon seedling wheat plants grown in solutions. The compounds employed were, unless otherwise stated, the purest preparations obtainable upon the market.

The water used in making solutions was the ordinary laboratory distilled water treated with carbon black. This treatment makes the distilled water a good medium for plant growth, as was demonstrated in Bulletin 36 of this Bureau.

The experiments upon the action of various organic compounds upon plants were conducted with the pure preparations dissolved in carbon-treated distilled water. Whenever possible, the highest concentration employed was 1,000 parts per million.

The concentrations of the individual compounds were usually 1,000, 500, 250, 100, 50, 25, 5, and 1 part per million, but a number of the compounds used were soluble only in quantities less than 1,000 parts per million, and in these cases the range of concentrations was much smaller. Such instances are noted in the last column of Table I.

The wheat was germinated according to the method described by Livingston,^a and in Bulletin 40 of this Bureau. The seedlings were put into the cultures at the stage when the first true leaf was beginning to emerge from its sheath. Salt-mouth bottles, having a capacity of 250 c. c., were used as culture jars, and ten wheat plants were grown in each jar.

Twenty wheat plants were used in testing each concentration and comparison was made always with an equal number of plants growing in carbon-treated distilled water under like conditions. During the seasons of the year in which the conditions for growth were the best, each experiment was continued for from eight to ten days, but during the cloudy winter weather some of the experiments were continued for twelve or fourteen days; the exact length of time for each experiment is given in the third column of Table I.

The experiments were always set up in duplicate under such conditions that individual variations could be eliminated. A brief survey of the plan of the experiments makes it evident, furthermore, that each culture in any given series constituted a control for the other cultures of that series. In addition each series was repeated several times, always in duplicate, according to the plan of procedure used for the first series. It is obvious that in this way each fact is really established by a large number of properly controlled experiments and that the separate experiments become, on this account, a control one upon the other, thus forming a network of experiments, all showing the same general action.

^a Livingston, *Plant World*, 9, 13 (1906).

Two, and sometimes three, criteria of growth were employed, viz, transpiration, green weight, and turgidity. No one of these criteria can be regarded as absolute, but they usually agreed in indicating the order of results, which was the point sought for in these experiments.

Transpiration, since it is proportional to the growth of leaves and roots, is a criterion capable of quite general application. The close relation between transpiration and the other reliable methods used for measuring growth has been shown by the investigations of Livingston ^a and Jensen. ^b

The weight of the tops or of the entire plants in the fresh condition was also taken as a measure of their growth and as a means for comparison with the control plants.

The condition of the root tips was also noted as an aid in determining the lethal concentration. When killed the root tips lose their turgidity and soon become slimy. Before this ensues they generally show indications of injury by discoloration in the region occupied by the most actively growing cells, or meristem. In some solutions the tips stop elongating and become swollen, simultaneous with more or less discoloration.

The photographic reproductions, Plates I to VI, show in a general way the relative growth of the plants in comparison with their controls.

Many of the substances employed produced an effect mainly upon the tops, others affected mainly the roots of the plants. In computing the effects of these different treatments, the transpiration figures are believed to be a more reliable indication of growth of wheat in water cultures, as far as the entire plant is concerned, than the weight of the green tops. Transpiration is more nearly proportional to the growth of both roots and tops; while the green weight is not at all an indication of root growth, and is moreover liable to some error in indicating the actual growth of tops.

It is not to be inferred, however, that any one standard is an absolute measure of growth of wheat in water cultures, but when only one is used, transpiration appears to be much more accurate than the green weight of tops.

COMPOUNDS ARISING FROM PROTEIDS.

In the breaking down of proteid bodies there are formed a number of amido acids as intermediate products, which are more or less unstable. These substances not only appear in the decomposition of plant remains, but exist in very considerable quantities at the time of germination and in the seedling plant. These bodies are found in relatively large amounts in plants especially in members of the legume and mustard families, not only in the seedling stages

^a Livingston, B. E., Bot. Gaz., **40**, 178 (1905).

^b Jensen, G. H., Bot. Gaz., **43**, 11 (1907).

of the plants, but also in the roots, rootstocks, stems, buds, and in all parts which store up reserve material for the nutrition of the plant. Since plants of these families are extensively used in the practice of green manuring they may become important soil constituents.

According to Dojarenko,^a the amido-acid nitrogen forms a very considerable portion of the nitrogen in humus bodies (22 to 70 per cent). S. Suzuki,^b in studying the formation of humus, was able to show by the decomposition products obtained that proteid bodies were present. He was able to show the presence of alanine, leucine, amino-valerianic acid, proline, aspartic acid, glutaminic acid, tyrosine, and histidine among the decomposition products obtained by treating the so-called humus precipitate with hot concentrated hydrochloric acid.

Aspartic acid ($C_4H_7NO_4$) has been found in young sugar cane and in the molasses of the sugar beet.^c It is probably not widely distributed in nature, but has been shown to exist in seedlings of the bean^d and pumpkin.^e

Aspartic acid was tested upon wheat seedlings, using concentrations ranging between 1 and 750 parts per million. The plants were killed in solutions stronger than 500 parts per million, presumably on account of the acid properties of the solution. In the solution containing 100 parts per million there was little injury, and below that no injury.

Asparagine ($C_4H_8N_2O_3$) is a water-soluble form of organic nitrogen material which is relatively abundant in plants. It was first found in the young shoots of asparagus and subsequently in a large number of other plants representing many different families.^f

^a Dojarenko, Landw. Vers.-Stat., **56**, 311 (1902).

^b Bull. Coll. Agr., Tokyo, **7**, 513 (1907).

^c Scheibler, Jahresb. Chem., 1866, 399.

^d Mercadante, Ber. chem. Ges., **8**, 823 (1875).

^e Schulze and Barbieri, Ber. chem. Ges., **11**, 710 (1878).

^f Delaville, Ann. Chim., **41**, 298 (1802).

Robiquet, jr., Ann. Chim., **55**, 152 (1805).

Vauquelin and Robiquet, Ann. Chim., **57**, 88 (1805).

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Schulze and Castoro, Zeit. physiol. Chem., **38**, 199 (1903).



FIG. 1.—EFFECT OF FRESH AND DISCOLORED SOLUTIONS OF TYROSINE.

[Wheat grown in: (1) Freshly prepared solution of tyrosine, 16 parts per million; (2) discolored solution of tyrosine, originally containing 16 parts per million.]



FIG. 2.—EFFECT OF CHOLINE, FORMED DURING THE GERMINATION OF SEEDS AND DECOMPOSITION OF VEGETABLE MATTER.

[Wheat grown in: (1) Control in pure distilled water; (2) solution of choline, 1,000 parts per million; (3) 500 parts; (4) 100 parts; (5) 25 parts; (6) 5 parts; (7) 1 part.]

Asparagine forms a good source of nitrogen for fungi,^a and bacteria.^b It is not toxic to higher plants in concentrations of 1,000 parts per million and less, which were used in our experiments. It appears to serve, as Nakamura^c has also shown, as a nutrient for the higher plants, although Klebs,^d found that a concentration of 1 per cent (10,000 parts per million) was sufficient to inhibit spore formation in a green alga.

Glycocoll ($C_2H_5NO_2$), amido-acetic acid, is one of the simpler proteid degradation products found where decomposition is occurring. It forms a moderately good source of carbon for bacteria and fungi,^e but a 1 per cent solution was found by Klebs^d to inhibit zoospore formation in the alga *Conferva minor*.

In our experiments with wheat seedlings glycocoll was beneficial to growth in a concentration of 1,000 parts per million and less.

Alanine ($C_3H_7NO_2$), or alpha-amidopropionic acid, is of interest because of its relation to phenylalanine and tyrosine, two substances which are found in many plants. Alanine itself does not appear to have been found as yet in plants; it is known, however, as a very common splitting product of proteids. A solution of alanine containing 500 parts per million was slightly injurious to the roots of wheat plants; in weaker concentrations no injury whatever was shown, and in fact growth was improved.

^a Mayer, Untersuch. über alkohol. Gärung, 1869.

Bokorny, Centr. Bakt. (2), **9**, 674 (1902).

A. Schulz, Just's Jahresb., 1877, 84.

Kusserow, Koch's Jahresb., 1897, 84.

Soldan, Koch's Jahresb., 1898, 82.

Laurent, Ann. Soc. Belge Micr., **14**, 29 (1890).

Nakamura, Bul. Coll. Agr., Tokyo, **2**, 468 (1897).

^b Eijkman, Koch's Jahresb., 1892, 71.

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Loew and Kozai, Bul. Coll. Agr., Tokyo, **5**, 137 (1902).

Sullivan, Jour. Med. Res., N. S., **9**, 109 (1905).

^c Bul. Coll. Agr., Tokyo, **2**, 466 (1897).

^d Die Bedingungen der Fortpflanzung bei einigen Algen u. Pilzen, Jena, 1896.

^e Loew, Chem. Energie d. lebenden Zellen, München, 1899, 69.

Laurent, Ann. Soc. Belge Micr., **14**, 29 (1890).

Tyrosine^a ($C_9H_{11}NO_3$) is one of the most important and most widely distributed proteid hydration products. Tyrosine exists as such in many plant organs, especially those which contain appreciable amounts of proteid, and is to be regarded as a splitting product of proteid. It has also been found in certain fungi.^b The proteids are bodies of exceedingly complex constitution and under the stress of artificial chemical treatment, or natural processes, the complex structure is partially broken down and the chief products of this splitting up of the molecule are amino-acids of the paraffin series, such as glycocoll, leucine, aspartic acid, glutaminic acid, arginine, histidine, etc., and compounds of the aromatic series, such as phenylalanine, tyrosine, etc. The tyrosine group appears to be present in the proteid molecule, and to it are due some of the reactions shown by this class of bodies.

Tyrosine in the culture bottles proved to have a very injurious effect upon wheat seedlings. At a concentration of 100 parts per million it killed the roots and injured the tops. It was likewise injurious in a concentration as low as 16 parts per million. During the

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- ^a For literature on tyrosine, see Reach, Virch. Arch., **158**, 288 (1899).
 Schulze, Barbieri, and Bosshard, Zeit. physiol. Chem., **8**, 63 (1884).
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 Bleunard, Compt. rend., **90**, 1080 (1880).
 Fleurent, Compt. rend., **121**, 216 (1895).
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 Schulze and Engster, Landw. Vers.-Stat., **27**, 357 (1881).
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 Bamberger and Landsiedl, Monatsh. Chem., **25**, 1030 (1904).
 Gonnermann, Arch. gesamt. physiol., **82**, 289 (1900).
 Planta, Ber. chem. Ges., **23**, 1699 (1890).
 Shibata, Jour. Coll. Sci., Imp. Univ., Tokyo, **13**, 329 (1900).
^b Winterstein, Zeit. physiol. Chem., **26**, 438 (1899).
 Winterstein and Hofmann, Hofmeisters Beitr., **2**, 404 (1902).
 Bourquelot and Harlay, Bul. Soc. Myc., **12**, 153 (1896).

first few days the roots showed a pathological condition, and before the tenth day they were entirely dead. At a concentration of 1 part per million there was a very slight increase in growth over the controls. The physiological action of different samples of tyrosine was not always of equal intensity.

Since tyrosine is so widely distributed in plants and results so readily from the decomposition of proteids, it is highly probable that it and its decomposition products are present in soils, at least as intermediary products of plant decay in the formation of humus. Especially interesting in this connection are the numerous products, such as the aromatic acids and phenols, which are so frequently found during the decay of the proteids, and all of which, as Baumann^a has shown, are probably derived from the tyrosine group in the proteid by oxidation and reduction processes together with the splitting off of water, carbon dioxide, or ammonia. Thus tyrosine ($C_9H_{11}NO_3$) by reduction can yield hydroparacumaric acid ($C_9H_{10}O_3$) and ammonia, and this in turn, by splitting off carbon dioxide, gives rise to p-ethyl phenol ($C_8H_{10}O$). By oxidation and splitting off of water this compound can yield p-oxyphenyl acetic acid ($C_8H_8O_3$), and on splitting off carbon dioxide, p-cresol (C_7H_8O),^b which on further oxidation and splitting off of water yields p-oxybenzoic acid ($C_7H_6O_3$), and then phenol (C_6H_6O) and carbon dioxide.

All these products except the p-ethyl phenol are known as albumin decomposition products. Phenyl propionic acid and phenyl acetic acid, which are likewise albumin decomposition products,^c can also result from tyrosine by successive degradation.

Bacteria seem to have the property of oxidizing tyrosine to ammonia, carbon dioxide, and water, as Hoppe-Seyler^d has only found these substances as end products in proteid decay under certain conditions, tyrosine as well as leucine appearing only as intermediary products.

In Bulletin 40 it was shown that toxic soil conditions could arise as the result of the direct functioning of the plant. Since we know that the metabolic processes of plants are greatly affected by different conditions, it seems reasonable that under such conditions the character of the toxic excretions might likewise be changed, and show therefore widely different properties.

It is well known that when seedlings are grown under different conditions the composition of the mixture of proteid degradation products is markedly changed. Under certain conditions the usu-

^a Ber. chem. Ges., **12**, 1450 (1879).

^b This has been shown to be toxic to lupines by True and Hunkel, Bot. Centr., **76**, 289 (1898).

^c Salkowski, Ber. chem. Ges., **12**, 648 (1879); Zeit. physiol. Chem., **9**, 491 (1885).
Kerry, Monatsh. Chem., **10**, 864 (1885).

^d Zeit. physiol. Chem., **8**, 214 (1884).

ally large accumulation of asparagine in legume seedlings has been found to give way to excessive formation of tyrosine and leucine. Bertel^a has shown that by excluding oxygen, and also under other conditions, larger amounts than are usually found of tyrosine accumulated in the cells of the roots.

These products of proteid degradation which occur in the processes of germination and plant growth are also largely affected by subsequent changes in the plants themselves, probably by the action of enzymes, oxidizing, and reducing the immediate degradation products of the albumin molecule, associated with a splitting of amido groups and carbon dioxide, giving rise to a very large number of possible decomposition products of these primary proteid degradation products. The study of the further changes which tyrosine can undergo in lupine seedlings by Bertel is a good illustration of such transformations. He demonstrated that the large accumulation of tyrosine in chloroformed seedlings disappeared after some time with the formation of homogentisinic acid and possibly its oxidation products, or at least with the formation of compounds giving reactions similar to those afforded by this acid. The proteid hydrolysis in plants is still too little understood to afford a clear conception of the processes which take place. Just what changes the primary hydration products undergo, and some of these changes are doubtless rapid, is but little understood, and it is evident that these metabolic processes of growing plants can not be studied without taking into consideration such secondary reactions as those mentioned above. It is to such secondary changes that the differences between the mixture of amino acids formed by artificial hydration processes from proteids and that found in plants must be ascribed. Tyrosine, which appears in the first stages of growth of the lupine seedlings in considerable amounts, is soon changed further by the enzyme tyrosinase, which is most plentiful in the upper portions of the roots. The homogentisinic acid, which results as the product of this reaction, accumulates according to Bertel under certain conditions, while under normal conditions further oxidation of this substance takes place in the youngest portions of the seedling roots.^b

The nature of this change, as well as the end products, is still unknown. It is interesting in this connection that in the course of physiological stimulation (hydrotropism, heliotropism, geotropism) there is an almost universal transitory checking of this further oxidation of homogentisinic acid, causing a temporary increase of the latter in the plant tissues.^c

^a Ber. bot. Ges., **20**, 454 (1902).

^b In this connection see Schulze, E. Ber. bot. Ges., **21**, 64 (1903); Schulze and Castoro, Zeit. physiol. Chem., **48**, 387 (1906).

^c Czapek, Ber. bot. Ges., **20**, 464 (1902); **21**, 229, 243 (1903); Jahrb. wiss. Bot. **43**, 419 (1906).

The action of tyrosinase on tyrosine, changing it to homogentisinic acid, carbonic acid, and ammonia with oxygen absorption, belongs probably to the most general processes of metabolism. Bacteria also produce tyrosinase, as was shown by Gessard^a for *Bacillus pyocyaneus*, and by Lehmann^b for other bacteria. Interesting in this connection is the appearance of guanidine in seedlings as found by Schulze^c which probably results, through the action of such secondary changes as those mentioned above, from arginine.

Upon long standing with exposure to air the tyrosine solutions appeared to have undergone this same change, all of them having become quite dark in color. The changes wrought were probably the same as those produced by the enzyme tyrosinase, which oxidizes tyrosine to homogentisinic acid which in turn gives rise to the dark-colored compound.

An instructive result was obtained by studying the action of such a tyrosine solution which had suffered discoloration incident to age. The solution had become so dark in color that it resembled manure extract. It was diluted so that it was equivalent to a 16 parts per million solution of fresh tyrosine. This solution was used for growing wheat seedlings in comparison with a freshly prepared solution of equal strength.

In a few days the plants in the two solutions showed pronounced differences, which became greater as the experiment was continued. Plate I, figure 1, shows the appearance of the plants when the experiment was discontinued at the end of the twelfth day. The plants in the fresh tyrosine were more poorly developed than those growing in distilled water. The leaves were narrow and beginning to die at the tips; the roots were dead and had become slimy. The plants in the discolored tyrosine had made excellent growth. They resembled plants grown in manure extracts. The leaves were rich, green, broad, and making vigorous growth. The roots showed a similar good development. During the course of the experiment the plants in the fresh tyrosine had transpired 19 grams of water and attained a green weight of 580 milligrams. The plants in the discolored tyrosine had in the same time transpired 38 grams of water and attained a green weight of 940 milligrams.

These results are believed to have importance in explaining the value and action of the so-called "green manures." As previously mentioned, tyrosine and related compounds are quite widely distributed in the vegetable kingdom. They may even increase in number

^a Compt. rend. Soc. Biol., **50**, 1033 (1898).

^b Münch. med. Wochenschr., **49**, 340 (1902).

^c Ber. chem. Ges., **25**, 658 (1892); Zeit. physiol. Chem., **17**, 193 (1892); Landw. Vers.-Stat. **46**, 383 (1895); Zeit. physiol. Chem., **30**, 241 (1901); Ber. bot. Ges., **21**, (1903).

and in quantity when vegetable matter is allowed to decompose in the soil. Our experiments indicate that bodies like tyrosine and choline have a toxic action upon plants, but that these substances in a more highly oxidized stage are actually beneficial to plant growth. When first applied to the soil, green manures may exert a depressing effect upon the growth of the crop. After time enough has elapsed for the decomposition of the vegetable matter with change to other compounds, the crop shows a marked benefit from the action of the green manure.

Leucine ($C_6H_{13}NO_2$), alpha-aminoisobutylic acid, is also a very common decomposition product of proteids. It also occurs in the fly agaric (*Amanita muscaria*), in vetches, lupine, gourd, molasses of the sugar beet, potatoes, blighted corn, etc. Laurent^a found that leucine was a favorable nutrient for fungi. The effect of leucine upon wheat plants was studied by allowing the plants to grow in solutions having a concentration of 500 parts per million or less. The growth of the plants was uniformly benefited by the presence of leucine.

COMPOUNDS ARISING FROM LECITHINS.

Choline^b ($C_5H_{15}NO_2$) is widely distributed in the vegetable kingdom, in seeds and the growing parts of plants. It is not related to the proteid bodies directly, but forms a part of the molecule of the organic

^a Ann. Soc. Belge Micr., **14**, 29 (1890).

^b Schulze, Zeit. physiol. Chem., **11**, 365 (1887); **12**, 405 (1888); **17**, 140, 193 (1892); Ber. chem. Ges., **22**, 1827 (1889); Landw. Vers.-Stat., **46**, 383 (1895).

v. Babo and Hirschbrunn, Ann. Chem., **84**, 22 (1852).

Claus and Keese, Jour. prakt. Chem., **102**, 24 (1867).

Schmiedeberg and Harnack, Jahresb. Chem., 1876, 804; Arch. exp. Path., **6**, 101 (1876).

Griess and Harrow, Ber. chem. Ges., **18**, 717 (1885).

Boehm, Jour. prakt. Chem., **138**, 37 (1884); Arch. exp. Path., **19**, 60, 87, (1885).

Jahns, Ber. chem. Ges., **18**, 2518 (1885); **26**, 1493 (1893); **23**, 2973 (1890); Arch.

Phar., **235**, 151 (1897); **229**, 675 (1891); **225**, 479 (1887).

Brieger, Zeit. physiol. Chem., **11**, 184 (1887).

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Schmidt, Arch. Phar., **229**, 467 (1891).

Herberger, Berzelius' Jahresb., **12**, 273 (1833). "Fagin" = choline.

Thoms, Ber. chem. Ges., **31**, 271 (1898).

Schulze and Frankfurt, Ber. chem. Ges., **26**, 2151 (1893).

Schulze, Frankfurt and Winterstein, Landw. Vers.-Stat., **46**, 383 (1895).

Kunz, Arch. Phar., **225**, 461 (1887); **223**, 721 (1886); **226**, 529 (1888).

Struve, Zeit. anal. Chem., **41**, 544 (1903).

Kunz-Krause, Arch. Phar., **231**, 613 (1893); **237**, 1 (1899).

Wenzell, Jahresb. Chem., 1864, 14.

Boehm and Külz, Arch. exp. Path., **19**, 403 (1865).

Boehm, Arch. Phar., **222**, 159 (1884).

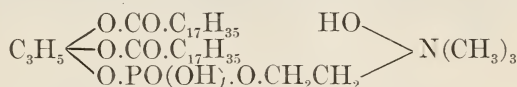
Hoppe-Seyler, Zeit. physiol. Chem., **2**, 427 (1879); **3**, 374 (1879).

Loew, Arch. gesamt. Physiol., **19**, 342 (1879).

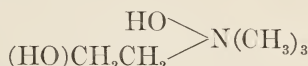
phosphorus compounds of both plants and animals, the lecithins, and is therefore always found as a product of the decomposition of these bodies. According to the observations recorded in the literature, choline is also found in various plant tissues associated with lecithin, and it does not follow that it must always be considered as a lecithin degradation product.^a

Struve^b has further distinguished between lecithin and water-soluble choline compounds and water-insoluble choline proteid bodies, the existence of which in plants has not yet been demonstrated.

The lecithins are closely related to the fats in constitution and are found in all vegetable tissues, being fully as widely distributed as the proteids and sugars, in all possible organs, and in every cell. According to Aso^c small quantities of lecithin are present in soils rich in organic matter. The lecithin molecule contains the grouping of palmitic, stearic, or oleic acid, and the grouping of choline. The constitution of stearin lecithin may be represented by the following formula:



and that of choline thus:



Choline can be prepared synthetically^d and is a sirupy liquid or highly hygroscopic crystalline mass of alkaline reaction. By boiling in aqueous solution it is decomposed into glycol and trimethylamine.

The lecithins are readily decomposed and split off choline and complex glycerophosphoric acids, which by further decomposition yield fatty acids, glycerin, and phosphoric acid. It is such a decomposition which takes place in germinating seeds and also in seedlings in the absence of light. During the process of germination comparatively large quantities of phosphoric acid are found in the water in which the seeds are lying, and this again disappears as the seedling begins to grow. This fact has repeatedly been demonstrated with germinating wheat in this laboratory, and the exudation of phosphoric acid by a large number of germinating seeds has also been shown by other investigators.

^a See especially Schulze, Zeit. physiol. Chem., **15**, 140 (1891), in this connection.

^b Ann. Chem., **330**, 374 (1903).

^c Bul. Coll. Agr., Tokyo, **6**, 277 (1904).

^d Wurtz, Compt. rend., **65**, 1015 (1868).

Schmidt and Bode, Arch. Phar., **229**, 469 (1891); Ann. Chem., **267**, 268, 300 (1891).

Kruger and Bergell, Ber. chem. Ges., **36**, 2901 (1903).

This liberation of phosphoric acid by germinating seeds is the direct result of such decomposition of the lecithins as described above. According to Zalewski,^a this decomposition of the lecithins is due to an enzyme. He has further shown that organic phosphorus compounds are quickly and almost totally decomposed by this enzyme during the process of germination, only 2 per cent remaining unattacked. The decomposition of lecithin by bacteria, with the formation of choline, has also been studied by Ruata and Caneva,^b and observations of choline in the presence of certain bacteria were also made by Schmidt.^c

Choline is formed as a product of this decomposition of the lecithins, and its wide distribution in the vegetable kingdom is therefore not at all surprising, and under certain circumstances the quantity liberated in this process may be, comparatively speaking, large.

The effect of choline on seedling wheat plants was therefore tried. The choline was obtained in a pure form (Merek's) and when introduced in varying amounts into water cultures of wheat it showed itself to be mildly toxic to wheat seedlings. The plants grown in this solution are shown in Plate I, fig. 2. In the lower concentrations, from 1 to 100 parts per million, the seedlings, more especially the roots, were stimulated to increased growth, behaving in this respect like other minute amounts of toxic materials and similar to the effect displayed when a toxic soil is treated with an absorbing agent or sometimes when merely diluted with water, as shown in a previous bulletin. Concentrations above 100 parts per million were distinctly toxic, producing a retardation in root and top development, although even a concentration of 1,000 parts per million was not fatal to the plants.

The almost universal distribution of choline directly in plant tissues and as the degradation product of the lecithins makes its presence in soils in which plants are growing and decaying highly probable, at least as an intermediary product of the changes which take place in the plant remains in the soil, and this has a still greater significance when we consider the closely related body neurine.^d Neurine ($C_5H_{13}NO$) is in all probability formed from choline or its mother sub-

^a Ber. bot. Ges., **24**, 285 (1906).

^b Ann. d'igiene sperim., **11**, 341 (1901).

^c Arch. Phar., **229**, 467 (1891).

^d Hofmann, Compt. rend., **47**, 558 (1858).

Liebreich, Ber. chem. Ges., **2**, 12 (1869).

Brieger, Ber. chem. Ges., **16**, 1186, 1405 (1883); **17**, 516, 1137 (1884).

Marino-Zucco, Ber. chem. Ges., **17**, 1043 (1884).

Hundeshagen, Jour. prakt. Chem., **136**, 245 (1883).

Gulewitsch, Zeit. physiol. Chem., **26**, 175 (1898).



FIG. 1.—EFFECT OF PIPERIDINE, FOUND IN PEPPER PLANTS AND OCCURRING IN MANY VEGETABLE POISONS LIKE ALKALOIDS.

[Wheat grown in: (1) Solution of piperidine, 500 parts per million; (2) 250 parts; (3) 100 parts; (4) 25 parts; (5) 1 part; (6) control in pure distilled water.]



FIG. 2.—EFFECT OF QUINONE, FOUND TO RESULT FROM THE ACTION OF A SOIL FUNGUS ON PROTEIDS.

[Wheat grown in: (1) Solution of quinone, 50 parts per million; (2) 25 parts; (3) 10 parts; (4) 1 part; (5) control in pure distilled water.]



FIG. 1.—EFFECT OF VANILLIN, FOUND IN VANILLA BEANS, LUPINES, BEETS, ROOTS, OATS, DECAYED WOOD, ETC.

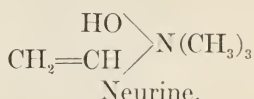
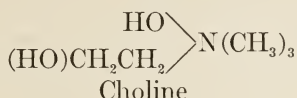
[Wheat grown in: (1) Solution of Vanillin, 1,000 parts per million; (2) 500 parts; (3) 100 parts; (4) 25 parts; (5) 1 part; (6) control in pure distilled water.]



FIG. 2.—EFFECT OF CUMARIN, OCCURRING IN TONKA BEANS, SWEET SCENTED GRASSES, SWEET CLOVER, ETC., AND FORMED BY THE ACTION OF MOLD FUNGI ON CARBOHYDRATES.

[Wheat grown in: (1) Control in pure distilled water; (2) solution of coumarin, 1 part per million; (3) 25 parts; (4) 50 parts.]

stance, lecithin, by the life processes of certain microorganisms,^a but has not yet been found in plants as such. By the action of dilute sulphuric acid lecithin is slowly decomposed, the choline under these circumstances being also readily changed to neurine. Neurine and choline can, moreover, be changed from one to the other by artificial processes. The close relationship of these two compounds is shown by the following formulas:



The neurine differs from choline by having one molecule of water less in its composition. Neurine belonging to the class of bodies known as ptomaines or toxins is very poisonous when introduced into the animal organism, and it was also found to be far more toxic to wheat seedlings in water cultures than was the choline.

A sample of neurine, prepared by Merck, was tested in water cultures with wheat seedlings in the same manner as choline. The lowest concentrations (6 parts per million) stimulated the growth, thus being analogous to the action of choline and toxic substances generally in low concentrations, but the plants were already greatly injured in a solution containing 25 parts per million, and a solution of 250 parts per million killed the seedlings in a few days. When the neurine was neutralized with acetic acid the same effects were obtained. It is thus evident that the neurine is very much more toxic than corresponding quantities of the higher oxygenated choline, which can even serve as a source of carbon for microorganisms. This toxicity of neurine to plants, in view of the fact that it can be formed under certain conditions by the action of microorganisms on the widely distributed choline and lecithin of plant remains, as well as by quite simple chemical reactions from choline, has considerable interest in connection with toxic soil conditions and the improvement following nitrification in such soils. The process of nitrification consists in the intermediary formation of nitrous acid. Neurine, as is readily seen from the formula, contains the vinyl group having a double linking of carbon atoms. Compounds of this nature readily add nitrous acid, forming very unstable and highly reactive nitroso derivatives which undergo further changes. It is possible, therefore, that if a body like neurine existed in a soil the nascent formation of nitrous acid might bring about a deep-seated change, rendering the toxic material harmless.

^aSchmidt and Weiss, Arch. Phar., 229, 481 (1891).

Betaine or oxyneurine ($C_5H_{11}NO_2$),^a which is closely related to both choline and neurine, is also found widely distributed in seeds and plants and can be derived artificially from choline by oxidation, with loss of water. In contrast with the toxic neurine, this oxyneurine was distinctly beneficial to the seedlings which grew well in concentrations ranging from 1,000 to 5 parts per million. It is classed by Loew^b among the compounds serving as a good source of carbon for bacteria and lower forms of plant life. It will be noted that this behavior of neurine, choline, and betaine toward wheat is in harmony with the results noted previously in this bulletin in the case of tyrosine or homogentisinic acid, namely, that the oxidized form of a toxic substance may be entirely harmless or even beneficial.

OTHER NITROGENOUS BODIES.

Urea derivatives.—Alloxan ($C_4H_2N_2O_4$), the ureide of mesoxalic acid, has not been reported as occurring in plants, although alloxantin, a product of the reduction of alloxan,^c has been prepared from convicine. This convicine is obtained from the seeds of *Vicia faba* and *V. sativa* and from beet juice.^d

Alloxan is stated by Loew^e to be an unfavorable source of carbon for bacteria. At the end of ten days the wheat plants which had

^a Ritthausen and Weger, Jour. prakt. Chem., **138**, 32 (1884).

Maxwell, Am. Chem. Jour., **13**, 469 (1891).

Scheibler, Zeit. Chem., 1866, 279; Ber. chem. Ges., **2**, 292 (1869); Ber. chem. Ges., **3**, 155 (1870).

Frühling and Schultz, Ber. chem. Ges., **10**, 1070 (1877).

Schulze and Urich, Landw. Vers.-Stat., **18**, 296, 409 (1875).

Andrlik, Velig and Staněk, Biochem. Centr., **1**, 303 (1903).

v. Planta, Ber. chem. Ges., **23**, 1699 (1890).

Orlow, Just's bot. Jahresb., 1897, II, 102; Chem. Centr., 1898, I, 37.

Naylor, Phar. Jour. Trans., **60**, 279 (1898).

Liebreich, Ber. chem. Ges., **2**, 13, 167 (1869); **3**, 161 (1870).

Husemann, Arch. Phar., **206**, 216 (1875).

Staněk, Chem., Centr., 1902, I, 1050; 1903, II, 24; Zeit. physiol. Chem., **47**, 83 (1906).

Willstätter, Ber. chem. Ges., **35**, 2700 (1902).

Andrlik, Chem. Centr., 1904, II, 309.

Griess, Ber. chem. Ges., **8**, 1406 (1875).

Marme and Husemann, Ann. Chem. Suppl., **2**, 383 (1863).

Marme and Husemann, Ann. Chem. Suppl., **3**, 245 (1864); Arch. Phar., **207**, 289 (1875).

Kraut, Ann. Chem., **182**, 180 (1876).

Schulze, Ber. chem. Ges., **22**, 1827 (1889); Zeit. physiol. Chem., **17**, 193 (1893).

Schmidt and Schütte, Arch. Phar., **229**, 523 (1891).

^b Loew, Bul. Coll. Agr., Tokyo, **2**, 51 (1894).

^c Ritthausen, Jour. prakt. Chem., **132**, 218 (1881); **167**, 487 (1899); **137**, 259 (1884); Ber. chem. Ges., **29**, 894, 2106 (1896).

^d von Lippmann, Ber. chem. Ges., **29**, 2653 (1896).

^e Loew, Chem. Energie d. lebenden Zellen. München, 1899, 69.

grown in the solution of alloxan containing 1,000 parts per million were dead, those in 100 parts per million were injured, while those in concentrations of 25, 5, and 1 parts per million were somewhat stimulated. Alloxan thus exhibits all the properties of a toxic substance in its action upon the growth of plants.

Guanine ($C_5H_5N_5O$), which is a constituent of guano, has also been found to be a product of the autolysis of yeast.^a It appears to be rather widely distributed and has been found in the seeds of vetch, alfalfa, clover, and gourd,^b in germinating barley,^c in sugar beet,^d and in sugar cane.^e Guanine is so slightly soluble in water that it was not possible to test its action in solutions having a concentration greater than 40 parts per million. Its effect upon wheat plants was tried in concentrations of 40, 20, 10, 5, and 1 part per million. In all these concentrations the guanine had a slightly beneficial effect upon the growth of the plants.

Xanthine ($C_5H_4N_4O_2$), which is closely related to guanine, has been reported as occurring in a number of plants.^f

Xanthine is so difficultly soluble in water that it was only tested in concentrations of 25, 10, 5, and 1 part per million. In all of these concentrations it exerted a slightly beneficial effect.

Guanidine (CH_5N_3) has been demonstrated in plants by Schulze^g and von Lippman,^h and according to the investigations of Kutscherⁱ guanidine may arise from the oxidation of arginine which is another proteid decomposition product found in plants. Interesting in this connection is the appearance of guanidine in seedlings, reported by Schulze, which probably results by further changes from arginine. Guanidine may be formed artificially by the oxidation of guanine. Guanidine was first shown to be toxic to plants by Kawakita,^j who found that a concentration of 2,000 parts per million guanidine hydrochloride was sufficient to kill young barley plants in three days.

^a Schutzenberger, Ber. chem. Ges., **1**, 192 (1869).

^b Schulze, Zeit. physiol. Chem., **9**, 420 (1885); **10**, 80, 326 (1886); Jour. prakt. Chem. (2), **32**, 433 (1885).

^c Ullik, Chem. Centr., 1887, 829.

^d von Lippmann, Ber. chem. Ges., **29**, 2645 (1896).

^e Shorey, Jour. Am. Chem. Soc., **21**, 609 (1899).

^f Salomon, Dubois' Arch., 1881, 161, 361; Bot. Jahresber. 1880 (1), 290.

Schulze, E., Landw. Jahrb., **12**, 909 (1883); Jour. prakt. Chem., **35**, 337 (1883); Landw. Vers.-Stat., **46**, 383 (1895).

Schulze and Steiger, Zeit. physiol. Chem., **9**, 43 (1887).

Menzio, Ber. chem. Ges., **21**, 619 (1888).

Baginsky, Zeit. physiol. Chem., **8**, 395 (1884).

von Lippmann, Ber. chem. Ges., **29**, 2645 (1896).

^g Zeit. physiol. Chem., **17**, 197 (1892).

^h Ber. chem. Ges., **29**, 2645 (1896).

ⁱ Zeit. physiol. Chem., **32**, 413 (1897).

^j Bul. Coll. Agr., Tokyo, **6**, 182 (1904).

He also found that while fungi were able to utilize guanidine hydrochloride as a source of nitrogen they were unable to use it as a source of carbon.

Guanidine carbonate in distilled water was tested on wheat plants in concentrations of 1,000 parts per million and less. The plants were killed in solutions of 100 parts per million and stronger in nine days. In all the lower concentrations, including 1 part per million, the wheat plants were seriously injured.

Skatol (C_9H_9N) is a common product of putrefaction of proteid materials and is known to be the product of the activities of certain bacteria. In solutions skatol proved to be somewhat toxic to wheat plants. A concentration of 200 parts per million was sufficient to kill seedling plants in nine days, and in the same time a concentration of 50 parts per million was injurious. In those concentrations where skatol had a harmful effect the roots of the wheat plants were more injured than the tops.

Pyridine (C_5H_5N) forms the nucleus upon which are built many of the alkaloids. It is obtained chiefly from coal tar, but may also be obtained from a number of alkaloids, such as nicotine, trigonelline, sparteine, and cinchonine, when these are highly heated, treated with alkalies, or distilled with zinc dust. Pyridine is a colorless liquid of penetrating odor and basic properties.

The action of pyridine and its homologues has received added importance for agriculture as a result of the investigations of Shorey,^a who obtained pyridine by the dry distillation of soil. The author cited says (p. 37): "As the fat in this soil was found to be only 0.005 per cent, it does not seem likely that the pyridine formed was due to the formation of the pyridine ring by condensation, but rather that it exists already in some form in the soil." Pyridine was also obtained from the same soil after extraction with ether; hence it seems that the view taken is the correct one. The presence of pyridine-like substances in soils was suggested by Berthelot and André,^b who attempted to isolate the compounds which give the characteristic odor to soils.

Laurent^c found that a 1 per cent solution of pyridine was toxic to the fungi which he studied. It has been observed by Falkenburg^d that the vapor of pyridine and some of its homologues is poisonous to bacteria.

In the experiments with wheat seedlings it was found that pyridine although not sufficiently toxic at a concentration of 1,000 parts per

^a Shorey, Rept. on Agr. Investigations in Hawaii, 1905; Bul. 170, Off. Expt. Sta., U. S. Dept. Agr., 1906.

^b Berthelot et André, *Compt. rend.*, **112**, 598 (1891).

^c Laurent, *Ann. Soc. Belge Micr.*, **14**, 29 (1890).

^d Czapek, *Biochemie der Pflanzen*, II, 926.

million to kill wheat plants in nine days, was nevertheless very injurious, especially to the growth of the green parts of the plants. In a concentration as low as 50 parts per million the growth of the tops was inhibited and the leaf tips turned brown. In the lower concentrations there was no stimulation of growth.

Picoline ($C_5H_4N.CH_3$), or methyl pyridine, was toxic to wheat plants, but only killed in the concentrations of 1,000 parts per million, and did not cause injury below 500 parts per million. The injury seemed to be manifested by the tops more than by the roots, thus resembling the action of pyridine.

Piperidine ($C_5H_{11}N$), or hexahydropyridine, is known to occur in nature, for example, in the pepper plant, and forms the nucleus of many alkaloids. Solutions of piperidine killed and injured at a lower concentration than either pyridine or picoline. A sample of piperidine that was neutralized with acetic acid proved to be more toxic than the strongly alkaline piperidine itself. Piperidine seems to injure the roots more severely than the tops.

Quinoline (C_9H_7N) forms the nucleus of many alkaloids found in plants belonging to the families Rubiaceæ and Loganiaceæ. Falkenburg^a found that quinoline was toxic to bacteria in a concentration of 2,000 parts per million. Reference to Table I shows that quinoline was sufficiently toxic to kill wheat plants at a concentration of 500 parts per million. So low a concentration as 5 parts per million affected them injuriously in six days. None of the lower concentrations caused stimulation of growth.

Ricin is the poisonous (for animals) principle of the castor oil plant (*Ricinus communis*). Its chemical composition is unknown, but it probably is a toxalbumen. Bokorny^b found that ricin was very slightly toxic to algæ and infusoria. On account of its insolubility no tests could be made upon solutions having a concentration greater than 50 parts per million. The wheat plants were injured to a slight extent in solutions below 30 parts per million, but most of the injury found was in solutions containing 40 and 50 parts per million. The roots always showed greater injury than the tops.

Mucin has been isolated from various animal tissues, and in one instance from plants, viz, the yams, *Dioscorea japonica* and *D. batatas*.^c Mucin is comparatively insoluble in water, and hence a wide range of concentrations was not available for testing its properties upon plants. In a saturated solution there was practically no growth of the roots of the wheat plants. In a solution of 100 parts per million, which is somewhat below the point of saturation, the

^a Czapek, Biochemie der Pflanzen, II, 926.

^b Bokorny, Th., Arch. gesamt. Physiol., 64, 262 (1896).

^c Ishii, Landw. Vers.-Stat., 45, 434 (1894); Beihefte Bot. Centr., 6, 20 (1896); Bul. Coll. Agr., Tokyo, 2, 97 (1894).

plants were seriously injured, both in the growth of roots and of tops. At a concentration of 5 parts per million mucin had a stimulating effect upon the growth of wheat plants. Both roots and tops were better than those of plants grown in distilled water.

NONNITROGENOUS BODIES.

Pyrocatechin ($C_6H_4(OH)_2$), or *o*-dioxxybenzene, has been found in the sap of *Mimosa catechu*, in the green leaves and berries of *Ampelopsis quinquefolia*,^a in the sap of sugar beets,^b and in several species of willow.^c The characteristic black color which the leaves of certain species of willows assume upon drying is probably the result of the oxidation of the pyrocatechin through the activity of an oxidase. Pyrocatechin may be formed as a decomposition product from many aromatic vegetable compounds, and even from sugar. It was shown by Yabe to be fatal to yeast cells and bacteria when used in concentrations of 0.4 to 0.58 per cent.^d

The effect of pyrocatechin was harmful, though not as toxic as some substances tried. Like many other toxic agents it was stimulating at low concentrations. The solution of 1 part per million gave slightly better plants than the control in distilled water.

Arbutin ($C_{12}H_{16}O_7$), which is a glucoside of hydroquinone (para-dioxxybenzene) is widely distributed among plants, especially in the Ericaceæ, or heath family. Klebs^e found that a 0.5 per cent solution of arbutin inhibited zoospore formation in *Conferva minor*. In its effect upon wheat plants arbutin is quite similar to pyrocatechin. It causes injury at about the same concentration and exerts a stimulating effect in the lower concentrations.

Pyrogallol ($C_6H_3(OH)_3$) has been more or less extensively tried on wheat seedlings and its effects described in experiments reported in Bulletins 28, 36, and 40 of this Bureau. In solutions of greater concentration than 25 parts per million it is usually toxic to wheat plants, but is stimulating in low concentrations. It was oxidized by the roots of the plants growing in it.

Phloroglucin ($C_6H_3(OH)_3$), like pyrogallol, is not found as such in plants, but may be derived from several aromatic plant constituents. Its toxic properties have been described by Bovert.^f Reference to Table I shows that its toxic properties are quite similar to those of pyrogallol. In common with pyrogallol this substance was oxidized by the roots of the seedling plants which grew in it.

^a v. Gorup Besanez, Ber. chem. Ges., **4**, 905 (1871); Sitzungsab. med.-phys. Soc. Erlangen, **5**, 39 (1873).

^b Lippmann, Ber. chem. Ges., **20**, 3298 (1887).

^c Weevers, Jahrb. wiss. Bot., **39**, 229 (1903).

^d Yabe, Bul. Coll. Agr., Tokyo, **2**, 73 (1894).

^e Die Bedingungen der Fortpflanzung bei einigen Algen u. Pilzen. Jena, 1896.

^f Jour. prakt. Chem., **127**, 445 (1878); Ber. chem. Ges., **12**, 2012 (1879).

Vanillin ($C_8H_8O_3$), the aromatic principle of the vanilla bean, is methyl protocatechuic aldehyde. It is probably not found as such in living plants, but exists in the form of a glucoside, which breaks down into vanillin and a sugar when the plant organs are dried. The glucoside, which gives rise to vanillin, probably occurs in a large number of plants. Vanillin, or substances which give rise to it, has been reported in oats (seeds and roots),^a seeds of the white lupine,^b asparagus shoots,^c in raw beet sugar,^d in Ilex leaves,^e in dahlia roots,^f etc. According to von Lippmann vanillin seems to arise during the decay of wood under certain conditions.^g

Behrens^h reported that when the odorless sap of fresh vanilla leaves was boiled with dilute sulphuric acid a perceptible vanilla odor was detected. Busseⁱ has shown that the characteristic vanilla odor is obtained when the sap of the fruits of the vanilla bean (*Vanilla pompona*) is acted upon by emulsin. This would indicate that the plants contained a vanillin glucoside.

Vanillin solutions were shown by Klebs^j to be fatal to the alga, *Conferva minor*.

The solutions of vanillin were distinctly toxic to wheat seedlings in our experiments, 500 parts per million being sufficient to kill wheat plants in nine days. In all the stronger concentrations the roots were quickly killed and became slimy. Before death ensued the roots were able to oxidize part of the vanillin to a purple insoluble dye which was deposited upon the surface of the roots. Reference to Plate III, figure 1, will show that the roots of plants grown in concentrations greater than 100 parts per million are dyed a dark color as a result of the oxidation of the vanillin. The toxic effects of the vanillin were less marked upon the tops of the wheat plants than upon their roots, nevertheless the green weight of the tops in all solutions was less than that of the tops of control plants grown in distilled water. The green weight of tops of plants grown in a vanillin solution so dilute as 1 part per million was 91 per cent of the controls and their transpiration was 72 per cent of the controls.

In some later experiments a few instances were found in which vanillin exerted a stimulating effect upon root growth when used

^a de Rawton, Compt. rend., **125**, 797 (1897).

^b Campani and Grimaldi, Chem. Centr., 1888, I, 377.

^c von Lippmann, Ber. chem. Ges., **18**, 3335 (1885).

^d Scheibler, Ber. chem. Ges., **13**, 335 (1880); Lippmann, *ibid.*, 662.

^e Polenske and Busse, Arb. kais. Gesundheitsamt, **15**, 171 (1899).

^f Payen, Ann. chim., **24**, 209 (1823); von Lippmann, Ber. chem. Ges., **39**, 4147 (1906).

^g von Lippmann, Ber. chem. Ges., **37**, 4521 (1904).

^h Tropenpflanzen, **3**, 299 (1899).

ⁱ Zeit. Untersuch. Nahr. u. Genuss., **3**, 21 (1900).

^j Die Bedingungen der Fortpflanzung bei einigen Algen u. Pilzen. Jena, 1896.

in the lower concentrations. This may perhaps be regarded as a phenomenon commonly exhibited by the oxybenzenes and their derivatives.

Vanillic acid ($C_8H_8O_4$), a monomethyl-protocatechuic acid, may be formed by the energetic oxidation of the aldehyde vanillin, or of coniferin. The solutions of vanillic acid showed marked toxic properties toward wheat plants. As in the case of vanillin, the oxidizing power of the roots formed, in the higher concentrations, an insoluble purple-brown dye which was deposited upon the surface of the roots. (Plate V, fig. 2.) Wheat plants were killed in seven days in the solutions containing 100 parts per million of vanillic acid. At a concentration of 25 parts per million only the roots of the plants were poorer than the control plants. In concentrations of 5 parts per million and 1 part per million the roots and tops were all better than those of the control plants. It thus appears that the vanillic acid is less inhibitive of root growth than the aldehyde, vanillin; in this respect it resembles more closely the action of the oxybenzenes than does vanillin.

Quinic acid ($C_6H_7(OH)_4COOH$) is found in cinchona bark, always accompanying the alkaloid, quinine. It is also found in other rather distantly related plants; in beet leaves,^a in moor hay,^b in cranberry leaves,^b and in other plants.^c

By the action of mold fungi quinic acid is broken up into propionic acid, acetic acid, and formic acid.

In all except the more dilute solutions quinic acid was very toxic to wheat plants. At the end of ten days the entire plants in concentrations of 1,000 and 500 parts per million were dead; in 100 parts per million only the roots were dead. In concentrations below 50 parts per million the plants were as good as the controls grown in distilled water, and in the solutions containing 5 parts per million there was slight stimulation of growth.

Quinone, $C_6H_4O_2$, benzoquinone, may be prepared artificially from quinic acid or from hydroquinone. It is a compound of interest in the study of soil problems since Beijerinck^d has found that a soil fungus, *Streptothrix chromogena*, has the ability to form quinone from proteids.

Quinone has been shown to be toxic to yeast by Laurent^e and to the seedlings of various plants, algæ, and fungi by Furuta.^f The last named investigator found that in concentrations of 1,000 and

^a von Lippmann, Ber. chem. Ges., **34**, 1159 (1901).

^b Loew, O., Jour. prakt. Chem., **19**, 309 (1878); **20**, 476 (1879).

^c Huseman-Hilger, Pflanzenstoffe, **2**, p. 1399.

^d Beijerinck, Centralbl. f. Bakt., Abt. 2, **6**, 2 (1900).

Arch. Neerland. d. Sc. exactes et nat. (3), **3**, 327 (1900).

^e Laurent, Ann. Soc. belge. Micr., **14**, 29 (1890).

^f Furuta, Bul. Coll. Agr., Tokyo, **4**, 407 (1902).



FIG. 1.—EFFECT OF CINNAMIC ACID, FOUND IN CERTAIN PLANTS AND RELATED TO CUMARIN.

[Wheat grown in: (1) Control in pure distilled water; (2) solution of cinnamic acid, 1 part per million; (3) 25 parts; (4) 50 parts; (5) 100 parts; (6) 350 parts.]

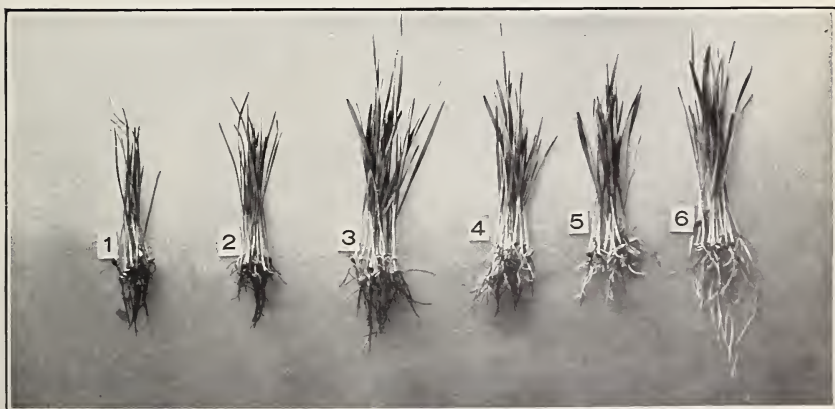


FIG. 2.—EFFECT OF ESCULIN, CLOSELY RELATED TO CUMARIN AND FOUND IN VARIOUS PLANTS.

[Wheat grown in: (1) Solution of esculin, 1,000 parts per million; (2) 500 parts; (3) 100 parts; (4) 25 parts; (5) 1 part; (6) control in pure distilled water.]



FIG. 1.—EFFECT OF HELIOTROPINE.

[Wheat grown in: (1) Solution of heliotropine, 1,000 parts per million; (2) 500 parts; (3) 100 parts; (4) 25 parts; (5) 10 parts; (6) 1 part; (7) control in pure distilled water.]



FIG. 2.—EFFECT OF VANILLIC ACID.

[Wheat grown in: (1) Solution of vanillic acid, 500 parts per million; (2) 250 parts; (3) 100 parts; (4) 50 parts; (5) 25 parts; (6) 5 parts; (7) 1 part; (8) control in pure distilled water.]

500 parts per million the roots of soy bean and wheat seedlings were injured within a few hours and killed in three or four days. Filaments of algæ were killed in a concentration of 1,000 parts per million in three or four hours and fungi in a somewhat longer period.

By reference to Table I (see p. 38) it will be seen that quinone was one of the most active poisons employed in the series of experiments. Wheat plants were entirely killed in solutions containing 100 parts per million and seriously injured in solutions containing 50 parts per million. In the lower concentrations, including 1 part per million, there was a slight inhibition of growth. No stimulation of growth was noticed in any of the concentrations tried.

Cinnamic acid is a phenol acid which is present in the bark of certain plants and forms esters which are found in the leaves of various other plants. Its effects upon the growth of seedlings was studied on account of its relation to cumarin. Cinnamic acid was found by True,^a to be fatal to the root tips of *Lupinus albus* in twenty-four hours when employed in a concentration of $\frac{m}{12800}$ (12 parts

per million). The same investigator also found that sodium cinnamate was toxic to root tips when employed in a concentration of $\frac{m}{800}$ (184 parts per million). Using a slightly different method and different plants, the results obtained with wheat seedlings varied, naturally, from those obtained with lupine. The same relations, however, hold true in both cases. Cinnamic acid is strongly toxic for seedlings, and its sodium salt is moderately toxic. Cinnamic acid is particularly toxic to the growth of roots. Reference to Plate IV, figure 1, shows that in all concentrations the growth of roots was inhibited. In fact it was only in concentrations weaker than 25 parts per million that there was any elongation of roots.

Cumarin ($C_9H_6O_2$) is a lactone of orthocumaric acid which may be regarded as oxy-cinnamic acid. Cumarin is a substance which has been found in a number of plants in different families of the plant kingdom. Among others, cumarin has been reported in the following plants: In grasses, viz, *Anthoxanthum* (Sweet Vernal), *Hierochloë* (holy grass), *Milium*, *Cinna*,^b in various dicotyledons, viz, *Herniaria*, *Ruta* (common beet), *Prunus mahaleb*, *Dipterix*, *Melilotus* (sweet clover), *Toluifera*, *Alyxia stellata*,^c the tubers of *Vitis sessi-*

^a Am. Jour. Sci. (4), **9**, 182 (1900).

^b Poulsen, Bot. Centr., **15**, 415 (1883).

^c Proc. Amer. Phar. Assoc. **36**, 581, (1888).

flora,^a *Galium triflorum* (bedstraw),^b *Asperula odorata* (woodruff), *Ageratum mexicanum*,^c and *Liatris odoratissima*.^d

Ortho-cumaric acid and o-hydrocumaric acid (Melilotic acid) are found in *Melilotus*, the sweet clover,^e in addition to coumarin.

Para-hydrocumaric acid, although it has not been reported in plants, is of interest on account of its relation to tyrosine. Through the action of bacteria, the amide group in tyrosine is broken up and ammonia liberated, thus giving rise to p-hydrocumaric acid. It is also of interest to note that Gosio^f has found that coumarin may be formed from carbohydrates by the action of certain mold fungi, e.g. *Aspergillus glaucus*, *A. varians*, *A. novus*, and *A. flavescens*.

Solutions of coumarin were shown to be toxic to the alga *Conferva minor* by Klebs,^g when used in strong concentrations.

The experiment showed that coumarin is extremely poisonous to wheat plants. At the end of five days the plants in 250 parts per million and stronger were dying, the roots and tops having made practically no growth. The root tips were swollen and slightly discolored, although the roots themselves were quite turgid. When the experiment was discontinued at the end of eight days the plants in the solutions of 100 parts per million were dead, although they had made a slight growth at the beginning of the experiment. The roots were discolored for a distance of 3 to 6 mm. from the tip and their surface was very slimy, due to the death of the outer layers of cells, which were then beginning to peel off. The leaves of the affected plants were short and broad, a feature not brought out by the illustrations, but which was very characteristic in all experiments with coumarin. The coumarin appeared to injure the meristematic tissue of the stem in such a way that only the first leaves were unfolded, and in most cases the sheathing leaf base was more or less swollen by the abnormal growth of the inhibited leaves within it.

The plants in the solutions of 50 parts per million were alive at the end of eight days, but all growth had ceased. The root tips were discolored and badly swollen. The leaves were dead for a considerable distance back from the tips. In the solution of 1 part per million the development of tops, as shown by the illustration (Plate III, Fig. 2), was practically equal to that of the control plants in distilled water. The root development was not as good, however, the tips being slightly discolored but not swollen.

^a Peckholt, Zeit. Allg. österr. Apothek.-Ver., 1893, 829.

^b v. Cotzhausen, Am. Jour. Phar., 48, 405 (1876).

^c Molisch and Zeisel, Ber. bot. Ges., 6, 353 (1888).

^d Vide Just's Bot. Jahresb., 1874, II, 947.

^e Zwenger and Bodenbender, Ann. Chem., 126, 257 (1863).

^f Atti R. Accad. Lincei, Classe sci. fis. mat. nat. (V) 15 (2) 59, (1906); abstr. Jour. Chem. Soc., 90, 699, (1906).

^g Die Bedingungen der Fortpflanzung bei einigen Algen u. Pilzen. Jena, 1896.

Daphnetin ($C_9H_6O_4$) which occurs in various species of *Daphne*^a is a lactone, and is to be regarded as (3, 4) dioxy-cumarin.^b

Daphnetin was relatively much less toxic than cumarin. On account of its insolubility it was impossible to test daphnetin in concentrations greater than 50 parts per million. At a concentration of 50 parts per million the green weight of tops produced in twelve days was 76 per cent of the controls. In concentrations of 25 parts per million and less the growth of tops was practically as good as in distilled water, and the development of roots was only slightly inferior.

Esculin ($C_{15}H_{16}O_9$) is of particular interest on account of its relation to cumarin. It is the glucoside of esculetin which is a lactone isomeric with daphnetin and shown by Tiemann and Will to be (4, 5) dioxy-cumarin.^c Both esculin and esculetin have been found in plants, but we know little of how widely distributed they are. Esculin as shown in Table I is decidedly less toxic to plants than cumarin, but more toxic than daphnetin.

A saturated solution of esculin was found by Klebs,^d to inhibit the formation of zoospores in *Conferva minor*, but it did not prevent the formation of chlamydozoospores in *Mucor racemosus*. Laurent found that a saturated solution of esculin was not inhibitive to growth of fungi, provided that glycogen were also present.^e

The esculin solutions had a blue fluorescence, when prepared; this was lost after plants had grown for a time in them. The roots which grew in the stronger solutions were colored dark yellow as a result of their oxidative activities, the dye formed being insoluble and remaining upon the surfaces of the roots where oxidation had occurred. This effect is shown in Plate IV, figure 2. The toxic effects of esculin were more marked upon the roots of the plants than upon the tops. In concentrations of 1,000 and 500 parts per million wheat plants were killed and were injured even in a solution containing 1 part per million. In the solutions containing 100 parts per million there was a distinct stimulation of the growth of tops.

Heliotropine ($C_8H_6O_3$) or piperonal is the aldehyde of piperonylic acid. It possesses the pleasant odor of heliotrope, and is, indeed, found in the flowers of *Nigritella suaveolens*.^f

^a Vauquelin, Ann. Chim., **84**, 173 (1812).

Zwenger, Ann. Chem., **115**, 1 (1860).

Russell, Rev. gen. Bot., **14**, 420 (1902).

^b Stunkel, Ber. chem. Ges., **12**, 109 (1879).

von Pechmann, Ber. chem. Ges., **17**, 929 (1884).

Gattermann and Kobner, Ber. chem. Ges., **32**, 287 (1899).

^c Ber. chem. Ges., **15**, 2073 (1882); **15**, 2106 (1883).

^d Die Bedingungen der Fortpflanzung bei einigen Algen u. Pilzen. Jena, 1896.

^e Laurent, Ann. Soc. Belge Micr., **14**, 29 (1890).

^f von Lippmann, Ber. chem. Ges., **27**, 3409 (1894).

The growth of wheat plants in solutions was markedly affected by the presence of heliotropine, although the plants were not killed by a concentration of 1,000 parts per million or less. It is worthy of remark that the tops of the wheat plants were more affected by the heliotropine than the roots. (See Plate V, fig. 1.) The roots were healthy and of nearly equal development in all solutions of heliotropine used.

TERPENE BODIES.

Borneol ($C_{10}H_{18}O$), an alcohol of the terpene series, may be regarded as a secretion of the Borneo camphor tree. From the fact that it is known as a plant secretion, as well as from its relation to camphor, its effects upon wheat plants are of interest. It is noteworthy in this connection that borneol acetate is an important constituent of the aromatic oils of many plants. It occurs, for example, in the needles of different species of pines, firs, spruces, and hemlocks, in golden rod (*Solidago canadensis*), in *Satureja thymbra*, in thyme, etc. The presence of borneol or some of its compounds in soils is not, therefore, unlikely, especially in the vicinity of coniferous trees. Although borneol is markedly insoluble in water, the weak concentrations obtainable were very toxic to both tops and roots of wheat plants. In ten days the plants were killed by a concentration of 100 parts per million and injured by 1 part per million.

Camphor ($C_{10}H_{16}O$), the ketone of borneol, is secreted by different plants, and is notably found in the wood of Cinnamomum, sassafras leaves, cinnamon root, spike, rosemary, basilicum root, etc. The poisonous effects of camphor upon seedlings have long been known. A list of some of the more important works is given below.^a

The action of camphor on wheat seedlings in our experiments was quite similar to that of borneol. A concentration of 300 parts per million was fatal to the seedlings in eight days, and 5 parts per million produced marked injury.

Terpene containing oils, such as turpentine oil ($C_{10}H_{16}$) are substances to be regarded as excretions of living plants, and many are

^a Treviranus, *Physiol. d. Gewächse*, **2**, 726.

Conwentz, *Bot. Ztg.*, **32**, 401 (1874).

Heckel, *Compt. rend.*, **80**, 1170 (1875).

Wilhelm, *Einwirkung des Camphors auf die Keimkraft* (Ref. in *Just's Bot. Jahresb.*, 1876, 884).

Nägeli, *Theorie der Gärung*, München, 1879, 84-85.

Burgerstein, *Landw. Vers.-Stat.*, **35**, 9 (1888).

Frank, *Pflanzenkrankheiten*, Breslau, 1895. II Aufl., **1**, 330.

Mesnard, *Ann. Sci. Nat.* (7), **18**, 257 (1893).

Detto, *Flora*, **92**, 147 (1903).

Heller, *Flora*, **93**, 1 (1904).

known to have a toxic effect upon the growth of plants.^a Heller^b has made the interesting observation that plants which secrete terpenes are less susceptible to injury by them than are those plants in which terpenes do not occur.

The action of terpenes has added interest for the study of problems dealing with soil fertility from the fact that the washings from the bark of trees and leaves have been shown^c to be toxic to seedlings. These washings may contain terpenes or terpenelike bodies, and their retention by the soil in the vicinity of trees would constitute a factor of some importance.

In our experiments turpentine oil proved to be fatal to wheat seedlings in a culture containing 500 parts per million and produced injurious effects at a concentration of 10 parts per million.

^a Goldsobel, Just's Bot. Jahresb., 1877, 224.

Nobbe and Hänlein, Landw. Vers.-Stat. **21**, 437 (1878).

Schwartz, Just's Bot. Jahresb., 1881, I, 307.

Omeltschenko, Centr. Bakt., **9**, 813 (1891).

Chamberland, Ann. Inst. Past., **1**, 153 (1887).

Riedlin, Dissertation, München, 1887.

Cadeac and Meunier, Ann. Inst. Past., **3**, 317 (1889).

Bokorny, Arch. gesamt. Physiol., **73**, 555 (1899).

Effront, Compt. rend., **136**, 1556 (1903).

^b Flora, **93**, 1 (1904).

^c Bul. 36, Bureau of Soils, U. S. Dept. Agr., 1907.

TABULATION OF EXPERIMENTAL RESULTS.

A general summary of the results given in the preceding pages is presented in the following table:

TABLE I.—*Effect of various organic compounds upon the growth of wheat plants, with especial reference to their toxic properties.*

Compound.	Duration of experiment.	Lowest concentration causing death.	Lowest concentration causing injury.	Concentration causing greatest stimulation.	Remarks.
	Days.	P. p. m.	P. p. m.	P. p. m.	
Aspartic acid, $\text{HOOC} \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$.	10	500	100		Normal growth in concentration below 100 p. p. m.
Asparagine, $\text{NH}_2 \cdot \text{OC} \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$.	9				No injury below 1,000 p. p. m.
Glycocoll, $\text{CH}_2(\text{NH}_2) \cdot \text{COOH}$.	9				Tops of all plants good. Roots slightly injured at higher concentrations.
Alanine, $\text{CH}_3 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$.	10		500	25	Only roots were injured at 500 p. p. m.
Leucine, $\text{CH}_3 \cdot (\text{CH}_2)_3 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$.	9				No injurious action.
Tyrosine, $\text{C}_6\text{H}_5 \begin{array}{l} \text{OH} \\ \\ \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH} \\ \\ \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH} \end{array}$	11		10		
Choline, $(\text{CH}_3)_3\text{N} \begin{array}{l} \text{OH} \\ \\ \text{CH} : \text{CH}_2 \end{array}$	10		500	1	Roots affected more than tops.
Neurine, $(\text{CH}_3)_3\text{N} \begin{array}{l} \text{OH} \\ \\ \text{CH}_2 \cdot \text{CO} \end{array}$	9	250	25		
Neurine (neutralized), $(\text{CH}_3)_3\text{N} \begin{array}{l} \text{OH} \\ \\ \text{CH}_2 \cdot \text{CO} \end{array}$	8	250	25		
Betaine, $(\text{CH}_3)_3\text{N} \begin{array}{l} \text{O} \\ \\ \text{CH}_2 \cdot \text{CO} \end{array}$	9				No injury.
Alloxan, $\text{CO} \begin{array}{c} \text{NH} \cdot \text{CO} \\ \diagup \quad \diagdown \\ \text{NH} \cdot \text{CO} \end{array} \text{CO}$	10	1,000	100		
Guanine, $\text{NH} \cdot \text{C} \cdot \text{NH} \cdot \text{CO} \cdot \text{C} \cdot \text{NH} \begin{array}{c} \diagup \quad \diagdown \\ \text{C} \cdot \text{N} = \text{CH} \end{array}$	12				Insoluble above 40 p. p. m. No harmful effects.
Xanthine, $\text{CO} \cdot \text{NH} \cdot \text{CO} \cdot \text{C} \cdot \text{NH} \begin{array}{c} \diagup \quad \diagdown \\ \text{C} \cdot \text{N} = \text{CH} \end{array}$	10				No injurious action.
Guanidine, $\text{HN} : \text{C} \begin{array}{c} \text{NH}_2 \\ \\ \text{NH}_2 \end{array}$	9	100	1		
Skatol, $\text{C}_6\text{H}_5 \begin{array}{c} \text{C} \cdot \text{CH}_3 \\ \quad \diagup \\ \text{NH} \quad \text{CH} \end{array}$	9	200	50		Roots injured more than tops.
Pyridine, $\text{C}_5\text{H}_5\text{N}$.	9		50		In solutions of 50 p. p. m. and less the root growth was normal.
Picoline, $\text{C}_5\text{H}_4\text{N} \cdot \text{CH}_3$.	7	1,000	500	100	
Piperidine, $\begin{array}{c} \text{H}_2\text{C} \quad \text{CH}_2 \\ \quad \quad \\ \text{H}_2\text{C} \quad \quad \text{CH}_2 \\ \\ \text{NH} \end{array}$	7	250	25		
Piperidine (neutralized), $\begin{array}{c} \text{H}_2\text{C} \quad \text{CH}_2 \\ \quad \quad \\ \text{H}_2\text{C} \quad \quad \text{CH}_2 \\ \\ \text{NH} \end{array}$	7	100	25	1	
Quinolin, $\begin{array}{c} \diagup \quad \diagdown \\ \diagup \quad \diagdown \\ \text{N} \end{array}$	6	500	5		
Ricin,	10		40		Insoluble above 50 p. p. m.
Mucin,	10		100		Not tested in concentrations higher than 100 p. p. m.

TABLE I.—*Effect of various organic compounds upon the growth of wheat plants, with especial reference to their toxic properties—Continued.*

Compound.	Duration of experiment.	Lowest concentration causing death.	Lowest concentration causing injury.	Concentration causing greatest stimulation.	Remarks.
	<i>Days.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	
Pyrocatechin, $C_6H_4(OH)_2(1,2)$	12	500	25	1	
Arbutin, $C_{12}H_{16}O_7$	12	500	25	1	
Phloroglucin, $C_6H_3(OH)_3(1,3,5)$	13	500	25	1	
Vanillin, $C_6H_3 \begin{array}{l} \text{CHO} \\ \diagup \text{O} \cdot \text{CH}_3 \\ \diagdown \text{OH} \end{array}$	9	500	1		
Vanillic acid, $C_6H_3 \begin{array}{l} \text{COOH} \\ \diagup \text{O} \cdot \text{CH}_3 \\ \diagdown \text{OH} \end{array}$	7	100	25	5	
Quinic acid, $C_6H_7(OH)_4 \cdot \text{COOH}$	10	500	100		
Quinone, $C_6H_4 \begin{array}{l} \text{O} \\ \diagup \quad \diagdown \\ \text{O} \end{array}$	9	100	1		
Cinnamic acid, $C_6H_5 \cdot \text{CH} : \text{CH} \cdot \text{COOH}$	8	100	25		
Sodium cinnamate.....	12		100		Roots were stimulated in lower concentrations.
Cumarin, $C_6H_4 \begin{array}{l} \diagup \text{CH} : \text{CH} \cdot \text{CO} \\ \diagdown \text{O} \end{array}$	8	100	1		
Daphnetin, $C_6H_2 \begin{array}{l} \diagup \text{CH} : \text{CH} \cdot \text{CO} \\ \diagdown \text{O} \\ (\text{OH})_2 \end{array}$	12		50		Insoluble above 50 p. p. m. Roots somewhat injured.
Esculin, $C_{13}H_{16}O_9$	13	500	1		
Piperonal (heliotropinè)— $C_6H_3 \begin{array}{l} \text{CHO} \\ \diagup \text{O} \\ \diagdown \text{O} \end{array} > \text{CH}_2$	7	100	1		
Borneol, $C_{10}H_{17}(OH)$	10	100	1		
Camphor, $C_{10}H_{16}O$	8	300	5		
Turpentine, $C_{10}H_{16}$	8	500	10		

THE REMOVAL OR AMELIORATION OF TOXIC CONDITIONS.

As a result of the experiments upon the nature and action of the organic bodies studied, it becomes evident that in many respects their similarity to toxic soil conditions is more than casual. It therefore becomes of interest to study the effect of treatments which have been found materially beneficial to toxic soils and their aqueous extracts. Some of these treatments consisted in adding substances which might be regarded as plant nutrients; other treatments which were equally beneficial did not add anything in the nature of a plant nutrient.

THE EFFECT OF TREATMENTS WHICH ADDED NO PLANT NUTRIENTS TO THE SOLUTIONS.

The results of adding absorbing agents and of boiling.—Two of the most toxic substances used in experiments upon plants were guanidine carbonate and neurine. In diminishing their toxicity two treatments, viz, treatment with carbon black and boiling, which have been found

remarkably efficient^a in improving the extracts of toxic soils for the growth of plants, were employed.

The treatment with carbon black consisted in adding a small amount of well-washed carbon black to the guanidine and neurine solution, and shaking them for several minutes. At the end of twenty to thirty minutes the carbon black was filtered out and the solutions used as the basis of water cultures for growing plants. The neurine solution was boiled for a very few minutes in an ordinary flask and, when cool, used for growing plants. The distillate from neurine was prepared by distilling a known quantity of an aqueous solution containing 250 parts per million of neurine. After a portion had passed over the distillate was made up to the original volume of the neurine solution with distilled water and used in water cultures. The effect of these treatments upon the toxic solutions is shown in Table II.

The guanidine carbonate solution was so much improved by treatment with carbon black that a solution which had contained 100 parts per million of guanidine was no more toxic to wheat seedlings than an untreated solution containing 1 part per million. A solution containing 25 parts per million of guanidine after treatment with carbon black gave a better growth of plants than did distilled water.

The neurine solution was so much improved by treatment with carbon black that wheat seedlings grown in a solution which had contained 25 parts per million of neurine made as good growth as the controls grown in distilled water. The plants which grew in a 25 parts per million neurine solution treated with carbon black were even better than those grown in distilled water.

TABLE II.—*Effect of various treatments in altering the critical concentrations of the respective solutions.*

[Parts per million.]

No.	Treatment.	Concentration of the solutions at which the plants were—		
		Killed.	Injured.	Stimulated.
1	Guanidine carbonate.....	100	1	
2	Same, treated with carbon black ^a	500	100	25
3	Neurine (neutral).....	250	25	
4	Same, treated with carbon black.....	250		25
5	Same, 250 p. p. m., boiled.....	Normal growth.		
6	Distillate from neurine solution containing 250 p. p. m.....	Normal growth.		

^a The figures in series Nos. 2 and 4 represent the original concentration of solutions which were given the treatments designated, and do not necessarily indicate the concentration of the solution used for growing plants.

The toxic effects of neurine seem therefore to have been entirely removed by boiling or by distilling, as normal growth resulted in those solutions.

^a See Buls. 28, 36, and 40 of the Bureau of Soils, U. S. Dept. of Agr.

Without speculating too much upon the nature of the changes produced by these various treatments, it may be pointed out that they have decreased the toxicity of the two original solutions. The decrease in toxicity is very similar to that observed when extracts of infertile soils are given similar treatment.

The results of adding an organic body.—The beneficial action of certain organic substances has been pointed out in various experiments described in previous bulletins of this Bureau. It was shown, for example, that the addition of 50 parts per million of pyrogallol to an unproductive soil increased the growth of wheat plants 101 per cent. The addition of small amounts (2 to 10 parts per million) of pyrogallol or alpha naphthylamine to the extract of an unproductive soil also had a beneficial effect. Organic substances like the ones mentioned can not be considered as plant nutrients; even if they were, the small amounts added would be insufficient to account for the results produced. Their action may be upon the plant itself, causing it to resist the toxic action of the soil extract, or else these substances, being quite active chemically, act directly upon the toxic substances in the solution.

It became of interest, therefore, to test the action of pyrogallol upon some substance which was known to have a decidedly toxic action upon plants. Cumarin was selected as the substance to be tried, because it had a nearly neutral reaction in aqueous solution. Four series of cultures of wheat plants were set up in solutions containing 75, 50, 25, and 5 parts per million of cumarin, respectively. Half of the cultures received pyrogallol at the rate of 2 parts per million. The other cultures served as controls for comparison. The experiment ran from February 13 to February 23. The following table shows the relative growth of the plants as measured by transpiration during that period:

TABLE III.—*Growth of wheat plants in solutions of cumarin, with and without pyrogallol.*

[Growth measured by transpiration and expressed in terms of the growth of controls in distilled water.]

No.	Solutions.	Without pyrogallol.	With pyrogallol.
1	Controls, without cumarin.....	100	94
2	Cumarin, 75 parts per million.....	Dead.	Dead.
3	Same, 50 parts per million.....	36	82
4	Same, 25 parts per million.....	82	86
5	Same, 5 parts per million.....	93	116

These results make it almost certain that pyrogallol had affected the action of the cumarin. The addition of pyrogallol to distilled water was not beneficial to plant growth in this case; in fact, the growth was slightly depressed as the result of its presence. This result speaks against any direct value of the pyrogallol as a nutrient

substance. Nevertheless the growth of plants in the cumarin solutions was benefited in each case, and in the solution containing 5 parts per million of cumarin and pyrogallol the growth of plants was distinctly better than in distilled water. In fact, this result would seem to indicate that the pyrogallol had not only been able to overcome the bad effect of the cumarin, but also to convert it into something slightly but distinctly beneficial to growth.

The results of replanting the solutions.—It was shown in a foregoing section that oxidation incident to exposure to air changed tyrosine from a substance having toxic properties to one having beneficial action. From this fact it seemed possible that similar beneficial changes might be wrought in other substances.

The work of Raciborski,^a has shown that the roots of plants have very definite powers of oxidation, and further work in this laboratory has shown that the roots of wheat plants grown in water culture are capable of quite energetic oxidation. It therefore seemed possible that the solutions in which a crop of wheat seedlings had grown might be less toxic to a second crop because of the oxidation performed by the roots of the first set of plants.

Several solutions were replanted after the first set of plants had been removed. The first crops in the various series were allowed to grow twelve to fourteen days. The growth of the second set showed that in three of the five substances the point at which injury was first noted was lowered, but the concentration necessary to kill the plants had not been altered. The lowest concentrations at which injury was shown in the first and in the second sets of plants were as follows:

TABLE IV.—*Effect of plants in altering the concentrations at which injury was first shown in various toxic solutions.*

[Parts per million.]

No.	Solutions.	Lowest concentrations causing injury.	
		First crop.	Second crop.
1	Arbutin.....	25	500
2	Cumarin.....	1	100
3	Cinnamic acid.....	25	25
4	Sodium cinnamate.....	100	100
5	Vanillin.....	50	500

The figures in Table IV show that for certain substances the injurious qualities of the solutions were sensibly altered by the growth of the first set of wheat plants. The arbutin solution, which originally contained 500 parts per million, was so reduced in toxicity by the growth of the first set of plants that it was no more toxic than

^a Bul. Acad. Sci., Cracovie, Math. Naturwiss. Kl., 1905, 338, 668.

a fresh solution containing 25 parts per million of arbutin. When cumarin solutions were replanted the solution originally containing 100 parts per million was no more toxic than a freshly prepared solution of 1 part per million. The cinnamic acid solution showed no apparent improvement when used the second time. It was thought that this might have been due to the acid properties of the compound. The experiment was accordingly repeated with sodium cinnamate, which was found by True^a to be much less toxic to seedlings of *Lupinus albus* than the cinnamic acid. The sodium cinnamate was likewise less toxic to wheat seedlings, but the growth of the first set of wheat plants did not perceptibly alter the point of injury for the second crop. It would seem from this that the activities of the roots were not able to alter sufficiently the properties of cinnamic acid to change its toxicity to seedlings. The question whether the activities of the roots were not modified by the properties of cinnamic acid in such a way that the ameliorating powers were lost seems worthy of more careful studies than we have been able to give it. The toxicity of the vanillin solutions is likewise greatly reduced by the growth of a previous set of plants. A solution which had originally contained 500 parts per million of vanillin was no more toxic to the second set of plants than a freshly prepared solution containing 50 parts per million had been to the first set of plants.

The point may be raised, and with some propriety, that the first set of plants absorbed and removed some of the toxic substances from the solutions. While this may be true, it is not likely that the quantities absorbed were sufficient to account for such differences as were noted in the case of arbutin, the concentration of which was so altered that the action of a solution originally containing 500 parts per million was no more injurious than that of a freshly prepared solution containing 25 parts per million. This oxidizing power of roots, described in a previous section, moreover makes it likely that there is an actual chemical change involved as a result of the oxidation.

That the concentration at which injury was manifested was altered, while that at which death occurred was not, is in accord with the assumption upon which the experiment was made. The roots which grew in the stronger solutions were soon seriously injured; hence any power they may have possessed to oxidize or otherwise to ameliorate toxic conditions had a very limited time in which to act.

^a Am. Jour. Sci. (4), 9, 183 (1900).

THE EFFECT OF ADDING SUBSTANCES USED AS FERTILIZERS.

In previous publications of the Bureau of Soils it has been pointed out that the beneficial effects of fertilizers are not necessarily due to their content of plant nutrients. The same improvement of growth often results in toxic soil extracts given treatments which do not add any nutrient substances. There is also evidence which indicates that fertilizers may bring about an improvement in conditions by some action upon the substances which previously were detrimental to growth.

To study this matter further, experiments were made in which substances commonly used as fertilizers were added to solutions of toxic substances, which in themselves have a marked toxic action upon wheat, and the effect upon growth noted. Forty plants in four cultures were used for each concentration of the toxic agent employed. The fertilizer salt was added to two of the cultures and comparisons were made with the two which received no fertilizer material. Sodium nitrate and calcium carbonate (lime) were the two substances experimented with. The former is especially efficient in producing improved growth when added to toxic soil extracts. In fact, it produces increased growth in the majority of cases when applied to soils. Calcium carbonate as lime is likewise efficient in improving growth, and is found to be quite generally beneficial when applied with green manures, which are known to contain a variety of organic compounds, some of which have been shown in previous sections of this bulletin to be harmful to plant growth.

Tables V, VI, and VII show the comparative growth of wheat plants in various concentrations of the toxic substances and the effect of the fertilizer salts upon the same. The growth of control plants in pure distilled water is used as the basis for comparison and represented as 100 in each case. Further experiments with cumarin and arbutin were performed, using, however, a smaller range of concentrations, yet the results obtained confirm those in the tables. The cumarin concentrations of 100, 50, and 10 parts per million gave a relative growth respectively of "dead" 47 and 66 without nitrate and "dead" 53 and 105 with 100 parts per million of nitrate added. The arbutin concentrations of 500 and 100 parts per million gave respectively 23 and 41 without nitrates and 27 and 78 with nitrates. It is quite evident from a survey of the results of all these experiments that these fertilizer salts had a beneficial action when added to the toxic solutions, and that the effect was much more marked in the weaker solutions than in the stronger ones. The beneficial effect of calcium carbonate in the cumarin solutions is shown in Plate VI, figure 1. They also indicate that there has been an interaction between the toxic substance and the fertilizer salt added. It may be noted that one of the toxic

substances (vanillin) produced, in the absence of fertilizer salts, in the lower concentrations what is ordinarily interpreted as stimulation. The phenomenon of increased growth in the presence of weak poisons is quite general and has been worked out in much detail by Raulin,^a Richards,^b Ono,^c and others.

TABLE V.—*Effect of adding sodium nitrate to toxic solutions of vanillin.*

[Relative growth measured by transpiration.]

No.	Solution.	Without nitrate.	NO ₃ , 100 parts per million added.
1	Control in distilled water.....	100	289
2	Vanillin, 500 parts per million.....	25	34
3	Same, 100 parts per million.....	53	184
4	Same, 25 parts per million.....	80	238
5	Same, 10 parts per million.....	126	246
6	Same, 1 part per million.....	132	271

TABLE VI.—*Effect of adding calcium carbonate to toxic solutions of cumarin.*

[Relative growth measured by transpiration.]

No.	Solution.	Without carbonate.	Adding CaCO ₃ 2,000 parts per million.
1	Controls in distilled water.....	100	153
2	Cumarin, 100 parts per million.....	Dead.	Dead.
3	Same, 50 parts per million.....	Dead.	Dead.
4	Same, 25 parts per million.....	36	58
5	Same, 10 parts per million.....	74	127
6	Same, 1 part per million.....	97	166

TABLE VII.—*Effect of adding calcium carbonate to toxic solutions of vanillin.*

[Relative growth measured by transpiration.]

No	Solution.	Without carbonate.	Adding CaCO ₃ 2,000 parts per million.
1	Controls in distilled water.....	100	209
2	Vanillin, 500 parts per million.....	25	107
3	Same, 100 parts per million.....	53	127
4	Same, 25 parts per million.....	80	183
5	Same, 10 parts per million.....	126	184
6	Same, 1 part per million.....	132	201

There can be no question that the increased growth in the vanillin cultures of lower concentrations without fertilizers was caused by some stimulating action of the vanillin upon the functions of the plants. Turning now to the columns expressing the effect of the fertilizer salts upon the vanillin, no stimulating effect is noticed. None of the solutions containing vanillin plus a fertilizer salt pro-

^a Ann. Sci. Nat. Bot. (5), 11, 93 (1869).^b Jahrb. wiss. Bot., 30, 665 (1897); Bul. Torrey Bot. Club, 26, 463 (1899).^c Jour. Coll. Sci. Imp. Univ., Tokyo, 13, 141 (1900). Hattori, H., *ibid.*, 15, 371 (1901).

duced an effect upon growth greater than distilled water containing only the fertilizer salt. In other words, the stimulating effect of the toxic agent totally disappeared. It appears, therefore, that the fertilizer salts had an action upon the toxic organic substances ameliorating the conditions for the growth of plants.

Further evidence on the action of fertilizer salts was gained from the results of replanting the vanillin solutions. The vanillin solutions enumerated in Tables V and VII received a second set of wheat seedlings after the first had been removed. The water lost by transpiration was restored by adding distilled water. The plants were allowed to grow from February 20 to March 1. The behavior of the second set of plants indicates that the conditions for growth were on the whole even better than those existing during the growth of the first set, except in the solution originally containing 500 parts per million vanillin, in which the plants were killed, as in the first planting. The injurious effect of vanillin, as already pointed out, was primarily on the root development of the first crop, and this condition was largely ameliorated by the calcium carbonate and sodium nitrate. In the second crop the root system was, generally speaking, much improved in all three series, including the one without fertilizer salts.

In order to determine whether the growth of the plants was a correct indication of the presence of vanillin the solutions were submitted to a chemical examination.^a Fairly large quantities of vanillin were shown by this test to be present in the solutions containing no fertilizer salts and originally containing 500 parts per million and 100 parts per million vanillin, while traces only could be found in the solutions originally containing 25 parts per million and 10 parts per million. No vanillin could be demonstrated in any of the solutions in which calcium carbonate or sodium nitrate had been present. That there had been a diminution and even a total disappearance of the toxic substance can not be questioned, because the experiment was repeated several times, always with the same result.

An additional experiment will be described for the purpose of giving a direct comparison between the growth of the first and second set of plants in toxic solutions containing fertilizer salts. Three

^a The method used seems to have been first described by Moerk, *Am. Jour. Phar.* **63**, 572 (1892) and cited in *Zeit. anal. Chem.*, **32**, 242 (1893). It consists in decolorizing the vanillin solution (if necessary) with freshly precipitated lead hydroxide, then adding bromine water drop by drop until a slight excess is present. Ferrous sulphate is finally added until the maximum blue-green color is reached. The test was slightly impaired by the yellow color formed by the reagents in solution to which calcium carbonate had been added, but the presence of sodium nitrate does not interfere with the test. The color was not found to be strictly proportional, although the depth of color produced was approximately indicative of the quantity of vanillin present.

different solutions were employed in this experiment; the first contained 100 parts per million vanillin, the second 100 vanillin and 100 sodium nitrate, the third 100 vanillin and 2,000 parts per million calcium carbonate. A set of wheat plants was allowed to grow in these solutions for eight days. The growth of the plants was of the same general character as represented in corresponding treatments in Tables V and VII. After removing the plants from these solutions, the original volumes were restored by the addition of distilled water, to replenish that transpired by the first set of plants, and a second set of seedlings was installed. Nothing was added to the solutions except the distilled water. At the same time a set of plants was installed in a new set of solutions precisely similar in composition to the original set. Accordingly, the plants in the new solutions represented a first crop in toxic solutions containing fertilizers, and the replanted set represented a second crop in the originally similar solutions. The plants in these solutions, together with controls in pure distilled water, were allowed to grow ten days. The relative growth of the plants in the various solutions is given in Table VIII and the plants are represented in Plate VI, figure 2.

TABLE VIII.—*Relative growth of first and second sets of plants in solutions containing 100 parts per million vanillin, with and without fertilizer substances.*

[The numbers and order of the solutions correspond to those of the plants shown in Plate V, figure 2.]

No.	Solutions.	Relative transpiration.	Relative green weight.
1	First crop in vanillin solution.....	45	100
2	Second crop in vanillin solution.....	93	103
3	First crop in vanillin solution+NaNO ₃ , 100 parts per million.....	114	99
4	Second crop in vanillin solution+NaNO ₃ , 100 parts per million.....	190	135
5	First crop in vanillin solution+CaCO ₃ , 2,000 parts per million.....	141	111
6	Second crop in vanillin solution+CaCO ₃ , 2,000 parts per million.....	166	100
7	Controls in pure distilled water.....	100	100

It will be seen that the results of this experiment confirm those of preceding experiments in showing that toxic properties were ameliorated both by the action of plant roots and by the presence of fertilizer substances. Where the two agencies worked in conjunction, the growth of plants was the best. The second crop naturally showed the better growth in each case owing to the action of the plant roots and fertilizers during the growth of the first crop.

Another experiment upon the action of these fertilizers was performed using arbutin as the toxic substance and growing two sets of plants in the solutions. Arbutin is shown by the figures given in Table I to be decidedly toxic to wheat plants, killing at a concentration of 500 parts per million and injuring at 25 parts per million. As before, sodium nitrate and calcium carbonate were added to the solutions. The set of solutions receiving sodium nitrate was accidentally lost before it was chemically examined for arbutin, and

hence does not appear in the records of the second crop. The growth of the plants in this experiment is shown in Table IX, where the figures represent the relative transpiration. The first crop grew eleven days, the second crop ten days.

TABLE IX.—*Relative growth of wheat plants in arbutin solution with and without fertilizer substances.*

No.	Solutions.	Relative transpiration.	
		First crop.	Second crop.
1	Controls in distilled water.....	100	100
2	Arbutin, 1,000 p. p. m.....	Dead.	Dead.
3	Same, 500 p. p. m.....	Dead.	36
4	Same, 200 p. p. m.....	41	71
5	Same, 100 p. p. m.....	45	94
6	Same, 50 p. p. m.....	80	125
7	Same, 1,000 p. p. m. +calcium carbonate 2,000 p. p. m.....	31	37
8	Same, 500 p. p. m.+calcium carbonate 2,000 p. p. m.....	51	84
9	Same, 200 p. p. m.+calcium carbonate 2,000 p. p. m.....	56	74
10	Same, 100 p. p. m.+calcium carbonate 2,000 p. p. m.....	70	148
11	Same, 50 p. p. m.+calcium carbonate 2,000 p. p. m.....	92	154
12	Distilled water + calcium carbonate 2,000 p. p. m.....	109	147
13	Arbutin, 1,000 p. p. m.+sodium nitrate 100 p. p. m.....	33	
14	Same, 500 p. p. m.+sodium nitrate 100 p. p. m.....	53	
15	Same, 200 p. p. m.+sodium nitrate 100 p. p. m.....	72	
16	Same, 100 p. p. m.+sodium nitrate 100 p. p. m.....	83	
17	Same, 50 p. p. m.+sodium nitrate 100 p. p. m.....	91	
18	Distilled water+sodium nitrate 100 p. p. m.....	146	

It will be seen from these figures that the general order of results was the same here as in the preceding experiments where a second set of plants was grown in toxic solutions. The second crop was in all cases better than the first. In making the comparisons it is of course necessary to use the growth in the "replanted" distilled water as the basis of the comparison and not fresh distilled water, since all solutions used for second crops contained the waste products of the first crop. Where the calcium carbonate had been added to the lower concentrations of arbutin, the second crop was remarkably good. These results bear out the results of chemical tests to determine the presence of arbutin.

It was found that diazobenzene-sulphanilic acid reagent^a could be used as a test for arbutin. While it was only approximately quantitative it gave good indications of the quantities present.

(a) Pauly, Zeit. physiol. Chem., **42**, 508 (1904); **44**, 159 (1905), prepares the diazobenzene-sulphanilic acid in the following way: 2 grams finely pulverized sulphanilic acid are shaken up with 3 cubic centimeters water and 2 cubic centimeters concentrated hydrochloric acid, and this mixture is added in small quantities, within one minute, to a solution of 1 gram of newly prepared potassium nitrite in 1 to 2 cubic centimeters of water; after each addition the mixture is cooled. The sulphanilic acid passes rapidly into solution, and is soon replaced by a dense, white, crystalline deposit of diazobenzene-sulphanilic acid; after a few minutes the fluid part is sucked off and the crystals are then washed with a little water. In testing, sodium carbonate solution is added to the fluid under examination till the reaction is alkaline, and then 3 to 5 cubic centimeters of a freshly prepared solution of a few centigrams of diazobenzene-sulphanilic acid in soda solution.



FIG. 1.—EFFECT OF CALCIUM CARBONATE IN OVERCOMING THE TOXIC EFFECT OF CUMARIN.

[Wheat grown in: (1) Solution of cumarin, 25 parts per million; (2) 25 parts + calcium carbonate; (3) 10 parts; (4) 10 parts + calcium carbonate; (5) 1 part; (6) 1 part + calcium carbonate; (7) control in pure distilled water; (8) control in pure distilled water + calcium carbonate.]



FIG. 2.—EFFECT OF FERTILIZERS IN OVERCOMING THE TOXIC EFFECT OF VANILLIN.

[Wheat grown in: (1) Vanillin solution, first crop; (2) second crop; (3) first crop, vanillin solution + sodium nitrate; (4) second crop, vanillin solution + sodium nitrate; (5) first crop, vanillin solution + calcium carbonate; (6) second crop, vanillin solution + calcium carbonate; (7) control in pure distilled water.]

Pure standard solutions of arbutin are colored bright crimson by the addition of a few drops of diazobenzene-sulphanilic acid reagent. Very weak solutions of arbutin, after the growth of plants, are strongly tinged with yellow. The results of the chemical tests made after the growth of the first and second crops are given in Table X.

TABLE X.—*Arbutin remaining in solutions after growth of first and second crops of wheat.*

No.	Solutions.	Results of tests to indicate arbutin.	
		After first crop.	After second crop.
1	Originally containing arbutin, 1,000 p. p. m.	Abundant.	Moderate.
2	Originally containing arbutin, 500 p. p. m.	Abundant.	Moderate.
3	Originally containing arbutin, 200 p. p. m.	Moderate.	Weak.
4	Originally containing arbutin, 100 p. p. m.	Weak.	Weak.
5	Originally containing arbutin, 50 p. p. m.	None.	None.
6	Originally containing arbutin, 1,000 p. p. m. + CaCO_3 2,000 p. p. m.	Abundant.	Weak.
7	Originally containing arbutin, 500 p. p. m. + CaCO_3 2,000 p. p. m.	Moderate.	Weak.
8	Originally containing arbutin, 200 p. p. m. + CaCO_3 2,000 p. p. m.	Weak.	Trace.
9	Originally containing arbutin, 100 p. p. m. + CaCO_3 2,000 p. p. m.	Trace.	None.
10	Originally containing arbutin, 50 p. p. m. + CaCO_3 2,000 p. p. m.	None.	None.

An inspection of these results shows that the calcium carbonate has had the same action upon arbutin as the fertilizer salts used in the previous experiments had upon vanillin. There had been a disappearance of arbutin from the solutions in which plants grew, but much more had disappeared from solutions containing the fertilizer salt.

The question next arises as to how these toxic substances were caused to disappear. Three possibilities seem to present themselves: (1) The plants themselves absorbed part of the toxic substance and oxidized some of it; (2) the fertilizer ingredients had a chemical action upon the toxic substance; (3) both plants and fertilizer salts acted together, either directly or indirectly, upon the toxic substance.

The first possibility was tested by the experiments in which a second set of plants was grown in the toxic solutions. It was there shown that the plants did have an ameliorating action, although they were only able to remove entirely the toxic substances in the lowest concentrations.

The second possibility, viz, that the fertilizer substances had a direct action upon the toxic substance, was tested by the following experiment: A solution containing 100 parts per million of vanillin was prepared; a portion of it received sodium nitrate equivalent to 100 parts per million of NO_3 , and another portion received calcium carbonate at the rate of 2,000 parts per million. These solutions were then allowed to stand eight days. At the end of that time another set of solutions, exactly similar to the first, was prepared.

They were therefore compared by cultivating plants in them and using the growth of the plant as an indicator of the amount of toxic substance present in each solution. It was thought that any action the fertilizers might have had would be shown by the growth of the plants in these solutions. The results of the experiment are presented in tabular form in Table XI.

TABLE XI.—*Relative growth, measured by green weight, of wheat plants in solutions of vanillin treated with fertilizer salts at different times.*

No.	Solution.	Solutions prepared at time of planting.	Solutions prepared eight days before planting.
1	Controls in distilled water.....	100	100
2	Vanillin, 100 parts per million.....	85	70
3	Same, + NaNO ₃ , 100 parts per million.....	99	92
4	Same, + CaCO ₃ , 2,000 parts per million.....	111	170

It will be seen from these figures that the 8-day action of the salts on the solution is somewhat contradictory. The solutions to which nitrate had been added was certainly not improved by standing this length of time. Those to which calcium carbonate had been added were, on the other hand, quite appreciably improved by standing, thus suggesting that this substance aids independently in overcoming the toxic properties of the vanillin.

In regard to the third possibility it might be pointed out that the experimental data thus far presented go to show that plants themselves can by their growth ameliorate the toxic conditions slightly, also that certain fertilizer treatments may bring about some improvement due probably to direct action on the substance, but far greater beneficial effects are produced by the combined action of plant and fertilizer salt. In other words, the plant and the fertilizer ingredients working together are able to accomplish more in the way of destroying toxic substance than either can do working alone. That such a substance as vanillin is destroyed does not admit of doubt if any conclusion is to be drawn from the experiments previously recorded. Whether the destruction of vanillin as such took place in the solution or within the cells of the wheat plants was not ascertained, but it probably takes place in the solution. This may be safely inferred from the behavior of the plants. Vanillin itself has a very inhibitive action upon the growth of the roots of wheat seedlings in water culture (Plate III, fig. 1). All plants grown in vanillin solutions showed more harmful effects in the growth of roots than in the growth of tops. When, however, the fertilizer salts were added in sufficient amount to ameliorate distinctly the conditions

for growth, the improvement in root development was relatively as great or even greater than in that of the tops.

A large number of experiments showing the benefits derived from the action of fertilizers on soils have been presented in Bulletin 40 of this Bureau and will not be presented in detail here. The results of the experiments here mentioned indicate that the small yields of unproductive soils can be greatly improved by treatments which remove or destroy toxic substances in those soils. In the experiments in which succeeding crops were grown in the same soil it was shown that certain fertilizers may act very beneficially upon soils containing toxic materials excreted from the plant roots.

It has been pointed out that methods of cultivation which promote the aeration of the soil and the growth of micro-organisms, may aid in destroying deleterious organic substances. The undue accumulation of such substances can also be prevented by proper and systematic crop rotation. In all these practices it is known that the addition of suitable fertilizers plays an important part in bringing about the destruction of harmful organic substances and consequent improvement of soil conditions. In other words, the experiments which showed that unproductiveness arises from the presence in soils of the waste products of plants have been supplemented by other experiments showing that fertilizer salts can actually bring about or aid in the destruction of toxic organic substances. It seems reasonable, therefore, to conclude that the benefits derived from fertilizers may, at least in part, be due to their direct or indirect action upon toxic organic substances in soils.

Attention may be again called to the fact that pot experiments in which fertilizers were employed to overcome the effects of replanting similarly show that certain fertilizers are able to destroy the toxic waste products of plant growth and to improve soil conditions. It was shown in the experiments mentioned that the ameliorating power does not necessarily depend upon the amount of plant nutrients which a fertilizer contains.

Summing up the results of experiments upon the amelioration of toxic conditions it appears that there are numerous ways in which such unfavorable conditions may be overcome. The toxic solutions were markedly improved by treatments similar to those which benefit the extracts of unproductive soils. Treatment with absorbing agents or brief boiling was beneficial. The toxic solutions were greatly improved after one set of wheat plants had been grown in them. Undoubtedly there was some toxic material directly absorbed by the first set of plants, but the amount was entirely too small to account for the diminished toxicity of the solutions when the second set of plants was grown. The vanillin solution, for example, was so

reduced in toxicity that a solution originally containing 500 parts per million was no more toxic to the second set of plants than a solution of 50 parts per million was to the first. It has been found that an equal number of wheat plants can remove in a similar length of time not more than 30 to 50 parts per million of nitrates from solution and there is no reason to believe that toxic substances should be removed at a much more rapid rate. The oxidizing powers of the roots therefore appear to be able to act upon the toxic organic materials in such a way that their toxic properties are lost. The large amount of root surface which most plants possess makes this oxidizing power an important one in relation to soil conditions and especially in relation to the destruction of toxic conditions through crop rotation.

In the experiments described it was shown that while the plants alone and fertilizer substances alone were able to accomplish a partial destruction of the toxic substances the combined action of plants and substances ordinarily employed as fertilizers caused a much greater destruction of toxic material and consequent improvement in growth.

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